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EDITORIAL

O extraordinário progresso das ciências médicas nos últimos anos veio transformar completamente não só a formação do médico moderno, como também mudar a própria conceituação da medicina.

Tal evolução, exigindo uma atualização constante dos conhecimentos e a participação efetiva do médico na pesquisa, definiu e destacou a posição do especialista, obrigando-o a um aprendizado cada vez mais amplo das ciências básicas, de modo a permitir a adequada compreensão do funcionamento dos órgãos e a investigação dos mecanismos mais íntimos de seus distúrbios.

A fisiologia, a bioquímica, a histologia e a genética tornaram-se tão fundamentais à pesquisa do endocrinologista, que, creio, existirão casos em que êle terá dúvidas se ainda é endocrinologista ou se já se identifica mais como bioquímico ou citologista.

Na realidade, para o desenvolvimento de suas pesquisas, tal dúvida não terá importância, desde que o especialista não se esqueça de que as técnicas de que lança mão em suas investigações, e até mesmo essas próprias investigações, têm como objetivo final a melhoria da condição humana e a manutenção das condições de higidez, visando o estabelecimento de uma sociedade equilibrada e harmônica.

Ao iniciarmos nossa gestão, como Presidente da Sociedade Brasileira de Endocrinologia e Metabologia, cumpre-nos dizer aos endocrinologistas brasileiros que procuraremos incentivar

a pesquisa na especialidade e o estreitamento das relações entre o clínico e o pesquisador.

Nesse sentido, dois cursos serão organizados: o primeiro sôbre exploração funcional glandular e o segundo sôbre fisiologia endócrina.

Estamos certos de que a convivência entre clínicos, especialistas e pesquisadores durante êstes cursos trará benefícios a todos; seja pelo aprendizado de novas técnicas e por uma atualização em fisiologia endócrina, para uns, seja pela participação nos problemas nascidos com o contato direto e continuado com o doente, para outros.

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STEATORRHEA COMPLICATING DIABETES MELLITUS

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Disturbances of gastrointestinal function have frequently been observed in diabetes mellitus and atributed by many investigators to changes in gastrointestinal motility secondary to diabetic visceral neuropathy.

BERGE et al. (1) were the first to demonstrate the presence of steatorrhea in the diarrhea of diabetics. These AA. reported six patients whose symptoms of intractable diarrhea — with post-prandial and nocturnal exacerbations and fecal incontinence — were similar to those previously reported in cases of diabetic diarrhea in which steatorrhea had not been looked for or demonstrated. In all patients there was laboratory evidence of steatorrhea and in three cases in which nitrogen balance studies were performed there was also azotorrhea. In every patient there were signs of autonomic neuropathy and

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direct or indirect evidence against the existence of pancreatic insufficiency (chronic pancreatitis or neoplasia), sprue and other conditions associated with steatorrhea. They suggested that disturbances of the innervation of the gastrointestinal tract due to autonomic neuropathy might be the significant factor in the production of both diarrhea and steatorrhea.

However, the same AA. described two additional cases of steatorrhea associated with severe diabetes in the absence of diabetic neuropathy, although functionally significant visceral neuropathy involving only the intestinal tract could not be excluded. Attempts to alleviate the diarrhea by treatment with pancreatin, anticholinergic drugs, adrenal steroids and other drugs were unsuccessful in all patients.

Since that report other authors have reported steatorrhea in diabetic patients in which most of the recognized causes of steatorrhea were excluded and a relationship to diabetes has been admitted. Among these cases there are a few (2-4) in which the clinical picture, course and the favorable response to corticosteroid therapy are more suggestive of nontropical sprue than of diabetic steatorrhea as described by BERGE et al. (1).

Recently, GREEN et al. (5) reported four cases in which diabetes mellitus coexisted with nontropical sprue and, in one of these, peroral biopsy of the small intestine was performed. Unlike most patients with diabetic steatorrhea, the diarrhea, and presumably the steatorrhea, preceded the diagnosis of diabetes by several years despite little or no evidence of neuropathy. Finally, the clinical response to the standard regimen for sprue was striking. Therefore, the differentiation between the diabetic steatorrhea and nontropical sprue associated with diabetes mellitus is very important from the therapeutic and prognostic point of view. Furthermore, the specimen of intestinal mucosa obtained by peroral biopsy showed the histologic alterations described in nontropical sprue which were not noted in the pathologic study of the intestinal tract in diabetic diarrhea reported by BERGE, SPRAGUE and BENNET (6).

The purpose of the present paper is to discuss the differential diagnosis of the disorders which could produce stea-

torrhea in diabetic patients, including chronic pancreatitis and a patient with celiac disease and diabetes mellitus.

MATERIAL AND METHODS

Studies were carried out on 9 diabetic patients with steathorrhea. In three of them there was chronic relapsing pancreatitis, in one, nontropical sprue and, in the remaining, diabetes mellitus was present without apparent pancreatic insufficiency.

The clinical data are shown in tables 1, 2 and 3.

The laboratory findings (tables 4 and 5) were similar in both groups and only one patient in each had hypocalcemia. The diabetes control, as shown by the fasting blood sugar range, was dificult, the requirement for insulin rising and falling. In four of the 5 patients with primary diabetes mellitus there were clinical evidences for urinary infection and/or pyuria. However, none of the patients with diabetes associated with pancreatitis presented clinical and/or laboratory findings of urinary infection. All patients with chronic relapsing pancreatitis had roentgenographic evidence of pancreatic calcification (figure 1) and all of them were chronic alcoholics.

For differentiation of steatorrhea of pancreatic insufficiency from impaired absorption due to intestinal dysfunction, nitrogen (method of Kjeldahl (7)) and radioactive (method of Ruffin (8)) and chemical fecal fat (method of van de Kamer (9)) determinations were done (tables 6 and 7). In two of the 4 patients with steatorrhea associated with primary diabetes mellitus in which fecal nitrogen was measured, the results were in the normal range. However, the nitrogen content of the feces was strinkingly increased in the pancreatogenous steatorrhea cases.

As to the chemical fecal fat balance, only one of the patients with primary diabetes had a clear-cut increase in fecal fat, two other cases had the fat content in the normal range and the remaining patient a slightly increased level. The 3 patients with chronic relapsing pancreatitis had a greatly increased fecal fat excretion.

TABLE 1

CLINICAL DATA RELATING TO CASES WITH STEATORRHEA COMPLICATING DIABETES MELLITUS

Initials, age (years) and sex (male, female)	CASE 1 C.M., 17 - M	CASE 2 B.M.P., 26 - M	CASE 3 A.C., 27 - M	CASE 4 A.R.G., 25 - M	CASE 5 I.C., 40 - M
Total duration of diabetes (years)	2	6	8	3	8
Duration of diarrhea (years)	1	2	4	0.1	1
Remission	Yes	Yes	Yes	Yes	Yes
Nocturnal occurrence	Yes	Yes	Yes	Yes	Yes
Fecal incontinence	No	No	No	No	Yes
Post-prandial exacerbation	No	No	No	No	No
Associated abdominal Cramping	Yes	No	No	Yes	No
Severity of diabetes Grade (*)	4	4	4	4	4
Complication of diabetes					
Retinopathy	No	No	Yes	No	Yes
Nephropathy	No (**)	No (***)	Yes	No	Yes
Neuropathy	Yes	Yes	Yes	Yes	Yes
Peripheral neuropathy	Yes	Yes	Yes	Yes	Yes
Vesical disfunction	No	No	No	No	No
Impotence	Yes	Yes	Yes	Yes	Yes
Dyshidrosis	No	No	No	No	No
Vertigo and syncope	Yes	Yes	Yes	Yes	Yes
Orthostatic hypotension	Yes	Yes	Yes	Yes	Yes
Orthostatic tachycardia	Yes	Yes	Yes	Yes	Yes

(*) Grade 4 (severe diabetes, controled by diet — more than 40 units of insulin daily)
(**) Necropsy: renal arteriosclerosis.
(***) Biopsy: normal kidney.

TABLE 2

NEUROLOGIC MANIFESTATIONS IN CASES WITH STEATORRHEA COMPLICATING DIABETES MELLITUS

Neurologic manifestations	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
Romberg's sign	No	Yes	Yes	No	No
Appendicular ataxia	No	No	No	No	No
Hipoactivity of deep reflexes	No	Yes	Yes	Yes (*)	Yes
Pains	Yes	Yes	Yes	Yes	Yes
Paresthesias	Yes	Yes	No	Yes	Yes
Cutaneous sensations (hypo-esthesia)	No	Yes (*)	Yes (*)	Yes (*)	Yes
Impairment of vibration sense	No	No (**)	Yes (*)	Yes (*)	Yes (*)
Muscle tenderness	Yes	Yes	Yes	Yes	Yes
Muscular wasting (diffuse)	No	No	Yes (*)	No	Yes

(*) Inthe lower limbs only. (**) Normal thresholds ("bio-thesiometer").

TABLE 3

DIABETES MELLITUS ASSOCIATED WITH CHRONIC PANCREATITIS: CLINICAL DATA

Name, age (years) and sex (male, female)	CASE 1 M.C.M. 52 - M	CASE 2 L.G.C. 26 - M	CASE 3 P.Z. 59 - M
Total duration of diabetes (years)	3	2	6
Proceeding history of attacks of pain in the upper abdomen (years)	5	4	7
DURATION of diarrhea (years)	1	1	1
Remission	Yes	Yes	Yes
Nocturnal occurrence	No	No	No
Fecal incontinence	No	No	No
Post-prandial exacerbation	Yes	Yes	Yes
Associated abdominal cramping	No	No	No
Severity of diabetes grade (*)	3	3	3
Complication of diabetes			
Retinopathy	No	No	No
Nephropathy	No	No (**)	No
Neuropathy	Yes	Yes	Yes

(*) Grade 1 (very mild diabetes, controllable by omission of sweets). Grade 2 (mild to moderate diabetes, controlled by a low carbo-hydrate diet without insulin). Grade 3 (moderate to severe diabetes, requiring diet + insulin dosage of 40 units or less, daily). Grade 4 (the same as Table 1).

(**) Biopsy: normal kidney.

For further evaluation of fat absorption, I-131-labelled oleic acid (Raoleic Acid PX-131, Abbott) and triolein (Raolein PX-131-Abbott) were used (tables 6 and 7). The patients with pancreatic insufficiency like those with primary diabetes mellitus showed poor absorption of the triglyceride triolein, more marked in the former. However, the patients with pancreatic disease showed a normal absorption of fatty acid, as contrasted to the poor absorption demonstrated by the patients with steatorrhea complicating primary diabetes mellitus.

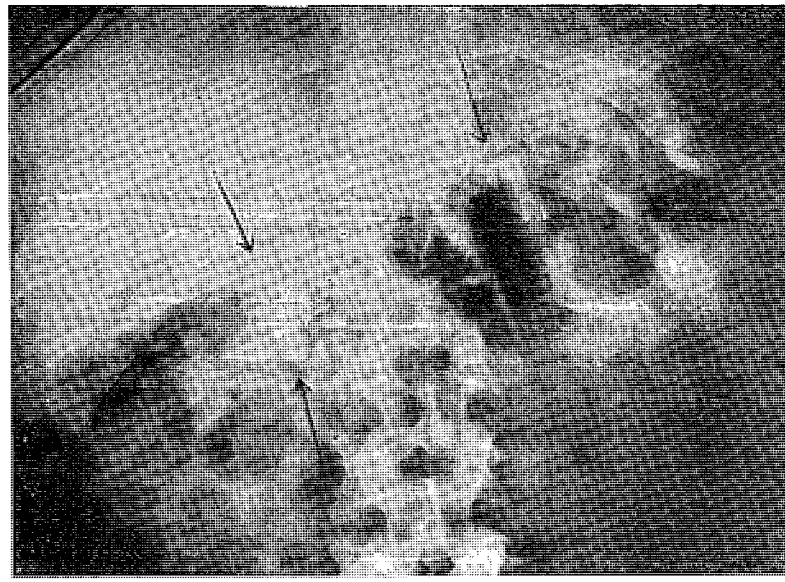
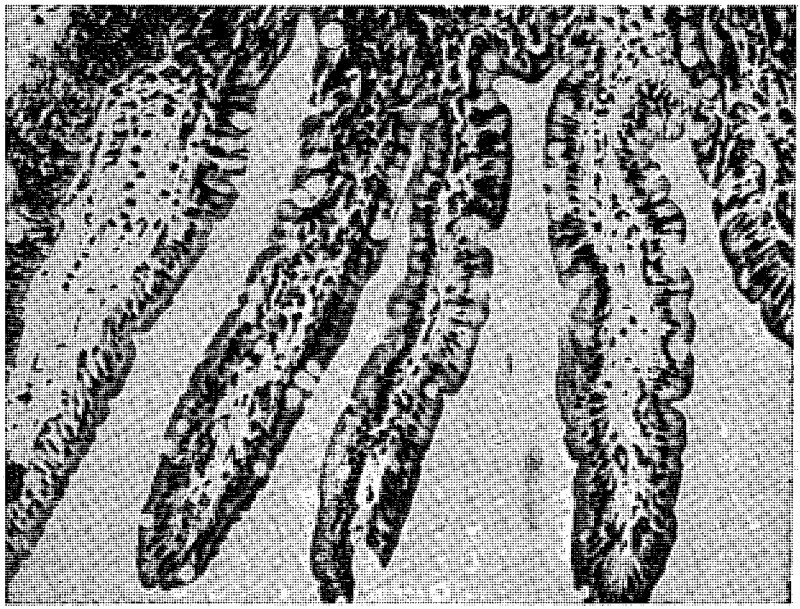
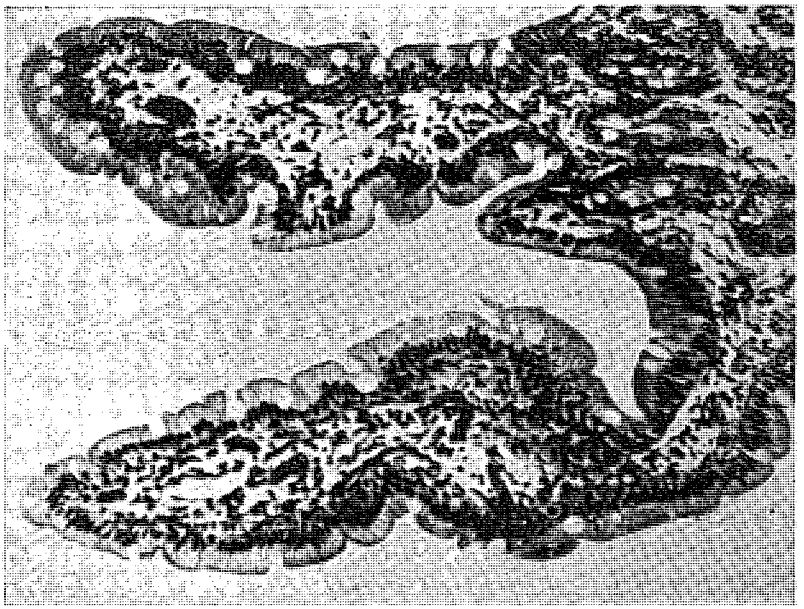


Figure 1 — X-ray of the abdomen showing pancreatic calcification in a patient with chronic relapsing pancreatitis (case 1).

The three patients with diabetes (table 6) in which the absorption of orally administered Cobalt-60 labelled vitamin B-12 — sp. activity — (Racobalamin PX-60, Abbott) was measured, a diminished urinary excretion was noted in the presence of normal gastric acidity. Unfortunately, similar studies were not performed in the chronic pancreatitis cases. Peroral jejunal biopsy was obtained in three patients with primary diabetes



B.M.P. (case 2)



A.C. (case 3)

Figure 2 — Peroral jejunal biopsy in cases of Idiopathic diabetes mellitus with steatorrhea (cases 2 and 3) showing no significant mucosal changes.

mellitus and in a case chronic pancreatitis, under fluoroscopic control using a CROSBY (10) capsule and no significant mucosal alterations were found (fig. 2 and 3).

The evaluation of pancreatic exocrine function was done utilizing secretin stimulation alone (11) or associated with pancreozymin (12). In the secretin test, volume and maximum bicarbonate concentration of samples of duodenal fluid was analysed by collection through a Wallace-Diamond double-lumen gastroduodenal tube, leaving its tip 40-50 cm. distal to the probable duodenal level. The duodenal aspirate was collected for four 20 minute periods.

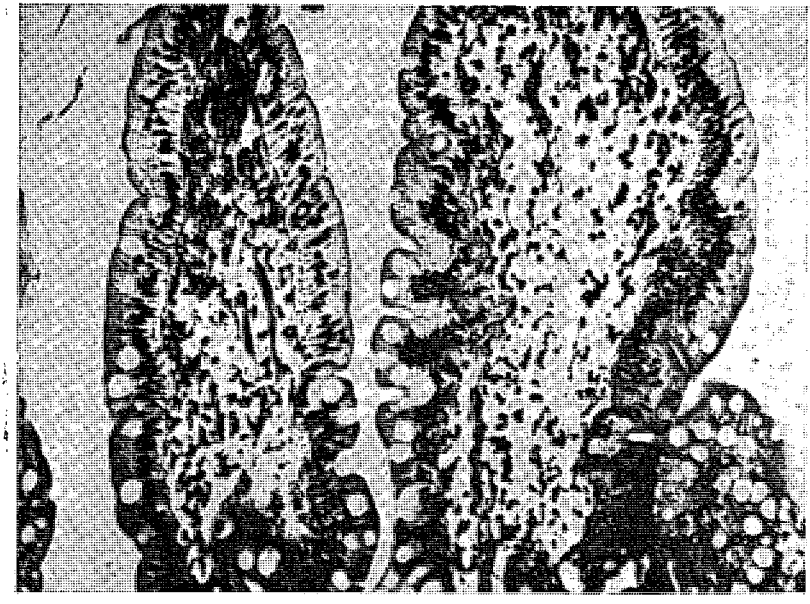


Figure 3 — Normal paror.l jejunal biopsy in a case of chronic pancreatitis and diabetes (case 1).

According to NEVES et al. (11), where the normal values correspond to a total volume of at least 76 ml with a maximum bicarbonate concentration above 65 mEq/L in one of the periods, our patients with steatorrhea complicating primary diabetes mellitus (table 8) showed a normal response to secretin. The patient with chronic pancreatitis demonstrated not only a re-

duction of volume of duodenal aspirate but also a decreased bicarbonate concentration (table 9) as might be expected.

In two diabetic patient with steatorrhea (table 8), the pancreozymin-secretin test, as suggested by SUN and SHAY (12), was normal and in case 3, in which only enzyme (amylase) concentration was measured, it was in the normal range. In

Figure 4 — Peroral jejunal biopsy of the patient T.P. (celiac disease), showing lymphocytic infiltrates of the mucosa and atrophy of the villi.

the patient with chronic relapsing pancreatitis (case 2 — table 9) a reduction in bicarbonate and amylase concentration of the duodenal aspirate was observed whereas the fluid volume was normal. This situation is frequently observed in the progressive destruction of pancreatic parenchyma (12).

DIABETES MELLITUS ASSOCIATED WITH CELIAC DISEASE

Case report: T.B., a 13-year old white girl, came to the Hospital das Clínicas in June 1962, complaining of diarrhea

TABLE 4

STEATORRHEA COMPLICATING DIABETES MELLITUS: General laboratory data

DETERMINATIONS	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
Blood studies					
Hemoglobin (g/100 ml)	15.0	14.5	11.4	12.7	11.8
Erythrocytes (cu. mm)	4.500,000	4.000,000	3.400,000	3.800,000	3.600,000
Macrocytosis	No	No	No	No	No
Range of fasting blood sugar (mg/100 ml)	55-476	48-630	52-436	116-396	60-412
Serum albumin/globulin (g/100 ml)	5.1/2.9	4.2/1.7	5.4/3.4	5.4/1.6	5.3/2.8
Serum calcium (mg/100 ml)			12.6	8.5	
Serum inorganic phosphorus (mg/100 ml)			3.7	3.8	
Serum amylase (normal: 100-300 units)		290		90	212
Blood urea (mg/100 ml)	30	25	38	30	58
Serum cholesterol (mg/100 ml)	268	155	186	201	180
Urinary studies					
Albuminuria (0 to ++++)	0	0	+	0	+
Pus cells and/or positive culture	Streptococcus	Pus-cells	Proteus		Pus-cells

TABLE 5

DIABETES MELLITUS ASSOCIATED WITH CHRONIC PANCREATITIS: general laboratory and X-ray data

DETERMINATIONS	CASE 1	CASE 2	CASE 3
Blood studies			
Hemoglobin (g/100 ml)	13.8	13.4	12.4
Erythrocytes (cu. mm)	4.000,000	4.100,000	3.700,000
Macrocytosis	No	No	No
Range of fasting blood sugar (mg/100 ml)	64-340	97-340	80-252
Serum albumin/globulin (g/100 ml)	5.7/2.6	7.7 (A+)	5.4/3.6
Serum calcium (mg/100 ml)	8.3	9.1	9.0
Serum inorganic phosphorus (mg/100 ml)	3.8	3.2	3.1
Blood urea (mg/100 ml)	21	19	36
Serum cholesterol (mg/100 ml)	201	173	110
Serum amylase (normal: 100-300 units)	159	80	70
Urinary studies			
Albuminuria (0 to ++++)	0	0	0
Pus cells and/or positiva culture	No	No	No
ROENTGENOLOGIC STUDIES			
Pancreatic calcification	Yes	Yes	Yes
Desmineralization of bone	Yes	Yes	Yes

TABLE 6

STEATORRHEA COMPLICATING DIABETES MELLITUS: intestinal absorption studies

Determination	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
Fecal fat (g/day-normal <7.0 g)	8.7	14.5	6.8	1.0	10.5
Fecal nitrogen (g/day-normal < 2.5 g)	1.9	3.4	2.5		3.1
I^{131}-labeled fat absorption tests					
Triolein test-fecal Radioactivity (% administered dose/24 hs — Normal<5%)	12.4	16.3	8.3	7.1	13.6
Oleic acid-fecal radioactivity (% administered dose/24 hs — Normal < 5%)	11.2	17.6	10.7	6.8	12.4
Co^{60} vitamin B_{12} test (schilling's test					
Urine radioactivity (% administered dose/24 hs — Normal: 10-30%)		1.9	5.8		4.11 + FI
Gastric acidity		Normal	Normal		Normal
Biopsy of small bowel		Normal	Normal		Normal

TABLE 7

DIABETES MELLITUS ASSOCIATED WITH CHRONIC PANCREATITIS: INTESTINAL ABSORPTION STUDIES

DETERMINATION	CASE 1	CASE 2	CASE 3
Fecal fat (g/day — normal < 7.0 g)	70.0	35.3	57.0
Fecal nitrogen (g/day — normal < 2.5 g)	8.0	4.2	7.0
I^{131}-LABELED FAT ABSORPTION TESTS			
Triolein test-fecal radioactivity (% administered) dose/24 hs — normal < 5%)	20.0	14.0	47.8
Oleic acid-fecal radioactivity (% administered) dose/24 hs — normal < 5%	3.6	4.8	1.9
Biopsy of small bowel		Normal	

TABLE 8

STEATORRHEA COMPLICATING DIABETES MELLITUS: DATA OF PANCREATIC SECRETORY FUNCTION

Response to secretin (4 periods of 20 min.)	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5
Volume (normal > 76 ml)		157	233	188	212
Maximal bicarbonate concentration (normal > 65 mEq/L)		72	72	80	78
Response to pancreozymin (10 min.)					
Volume (normal > 20 ml)				72	74
Maximal bicarbonate concentration (normal > 50 mEq/L)				88	82
Amylase concentration (normal > 70.000 units Somogyi/100 ml)			117.000	80.000	120.000

TABLE 9

DIABETES MELLITUS ASSOCIATED WITH CHRONIC PANCREATITIS: data of pancreatic secretory function

	CASE 1	CASE 2	CASE 3
Response to secretin (4 periods of 20 min.)			
Volume (ml — normal > 76 ml)	46	27	45
Maximal bicarbonate concentration — (normal > 65 mEq/l)	29.8	24.1	34
Response to pancreozymin (10 min.)			
Volume (normal > 20 ml)		50	
Maximal bicarbonate concentration — (normal > 50 mEq/l)		14.7	
Amylase concentration — (normal > 70,000 Somogy units/100 ml)		1350	

since birth with several bouts of dehydration. Her growth had always been deficient and at the age of 3 months the diagnosis of congenital bilateral cataracts was made. Since the age of 3 years the stools were described as watery, bulky and light in color. Initially, the diarrhea had occurred intermittently but for the last couple of years it had been continuous. Associated with this symptom there was abdominal cramping. One year prior to admisison the patient was found to have diabetes mellitus being initially treated with 30 units of NPH insulin and later on with chlorpropamide and DBI.

There was no familly history of diabetes. Two of her brothers had weighed more than 10 lb. at birth. An oral glucose tolerance test performed in the patient's mother was normal at the time of admission.

On physical examination the patient appeared intensely malnourished. Her weight was 23 kg. and her height 134 cm. Her blood pressure was 90/60 mm of mercury. Positive findings included subcutaneous hemorrhages at the level of the right thigh and both knees and gengival bleeding. The ocular examination demonstrated operated congenital cataracts (at the age of 3 years), bilateral nystagnus. The ocular fundi were normal. The abdomen showed slight gaseous distention. The neurologic examination was normal and hypertrophic osteoarthritis of the fingers was present. Positive laboratory findings at that time included normochromic anemia (9.9 g of Hemoglobin/100 ml.), micro and macrocytosis, normal platelet count, normal bleeding and coagulation times. The prothrombin time was greater than 100 seconds (1% of normal) and after I.M. vitamin K it became 12 seconds (100% normal) by Quick's method. The hematocrit was 38%. No albumin and sugar were present in the urine. Blood chemistries were: fasting blood sugar 96 mg/100 ml., blood urea 52 mg/100 ml., serum albumin 3.8 g/100 ml., globulin 1.7 g/100 ml., serum calcium 8 mg/100 ml., serum inorganic phosphorus 3.6 mg/100 ml., alkaline phosphatase 13.1 King Armstrong units, serum cholesterol 161 mg/100 ml. The microscopic examination of the stools did not show parasites or ova. The fecal fat excretion was 8.3 g/day, on admission, and decreased to 2.5 g per day

during remission of the steatorrhea. Gastric analysis was normal. The d-xylose absorption test whosed a 5-hour urinary excretion of 5.8% after an oral loading dose of 25 g (normal excretion: 18 ± 10.3%).

Sweat elctrolyte (sodium and chlorides) levels were normal. The biopsy of the jejunal mucosa showed the picture as described by RUBBIN et al. (13) in celiac disease (identical to non-tropical sprue) (fig. 4).

The patient was treated with vitamin and mineral supplements; a diet restricted in fat and residue and a change to NPH insulin (15 units daily) resulted in improvement of the patients condition but diarrhea persisted. However, when steroid treatment (6-methyl prednisolone) was started severe ketosis developed and the drug was stopped without evaluation of the possible effects on the diarrhea.

COMMENTS

Our material allows clear differentiation of the cause of steatorrhea when associated with diabetes mellitus: the analysis of tables 1 and 3 and the case of celiac disease shows that when chronic relapsing pancreatitis is associated with diabetes mellitus the historical evidence indicated that the onset of diabetes antedated the development of steatorrhea by 1 or more years. However, this relationship is inverse when the diabetes is associated with non-tropical sprue or celiac disease, as noticed in 3 of the 4 cases published by GREEN et al. (5). In case n. 4 of GREEN et al. (5) and those described by Ellenberg and Bookman (3) despite the occurrence of diarrhea years after the onset of diabetes mellitus, a diagnosis of sprue rather than of diabetic steatorrhea seemed to be justified by the absence of visceral neuropathy and by the satisfactory clinical response obtained on the gluten-free diet. Besides, the intestinal peroral biopsy in the 4th case of GREEN et al. (5) demonstrated the mucosal changes that have been described in sprue.

These cases show that sometimes the differentiation between diabetic steatorrhea and non-tropical sprue associated

with diabetes mellitus might be difficult and depend upon histologic study of the mucosa of the upper part of the small intestine.

As far as the differential diagnosis between diabetic steatorrhea and that from chronic relapsing pancreatitis associated with diabetes is concerned the latter always shows evidences of symptoms of pancreatitis (abdominal pain) preceeding the onset of diabetes by a period from 1 to 2 years, in our material. The analysis of Sprague's report (14) based on a study of twenty-four cases of diabetes associated with chronic relapsing pancreatitis seen in the Mayo Clinic from 1939 to 1945, shows that the time elapsing between the onset of symptoms of pancreatitis and the diagnosis of diabetes mellitus was less than a year in 5 patients and between 1 and 5 years in four.

This observation deserved special comment from the Author (14) because it is unusual for diabetes to make its appearance after such a brief history of pancreatitis. Among these five cases there were two in which a family history of diabetes casts some doubt on the assumption that the pancreatitis was the sole cause of the diabetes. However, none of our cases of pancreatitis had a family history of diabetes. It is not always possible, because of the virtual absence of pain, to be sure of the duration of pancreatitis, as it happened in two of Sprague's patients (14). Probably in our cases the process was severe enough to sufficiently damage the pancreas and to give rise to diabetes within a short period of time.

In relation to the characteristics of the diarrhea, the nocturnal exacerbation noticed in our patients with diabetic steatorrhea was similar to that described in the literature.

None of our patients with chronic pancreatitis showed nocturnal exacerbation of the diarrhea but post-prandial exacerbations were present. The latter was not observed in our primary diabetes mellitus patients in contrast to that described by BERGE (1).

Unlike the patients with diabetic steatorrhea who demonstrate visceral neuropathy, those with chronic pancreatitis only

show peripheral neuropathy. As previously observed by BERGE et al. (1) these patients usually show retinopathy and nephropathy (only one of our 4 cases and 3 out of the 6 cases described by BERGE et al. (1) did not demonstrate the above complications).

Similar to the reports in the literature, in the cases of steatorrhea complicating diabetes diabetes mellitus with neuropathy, the diabetes is severe and difficult to control. In the same way, once permanent diabetes from chronic pancreatitis had become established, it exhibited fluctuations in severity, the requeriment for insulin changing with the evolution of the pancreatic lesion. Probably, as mentioned by SPRAGUE (14), the flare-up of diabetes are due, not solely to diminished production of insulin by the pancreas during acute attacks, but also to a nonspecific aggravation of the disease such as may occur in any case of diabetes with almost any intercurrent illness, particularly a painful or febrile illness.

The correlation of radioactive and chemical fecal fat determinations clearly demonstrate that the fecal radioactivity, after the I-131-labelled oleic acid and/or triolein, was abnormaly high in all patients with diabetic steatorrhea. However, the chemical determination of fecal fat, after a constant ingestion of 100 g of fat daily, was abnormal in only two of our patients (table 6).

As for fecal fat the measurement of the nitrogen content of feces increased in only two of the 4 cases of diabetic steatorrhea. The 3 patients described by BERGE et al. (1) where nitrogen balance studies were performed had azotorrhea. On the other hand, there was a clear-cut increase in fecal nitrogen in pancreatogenous steatorrhea.

The study of absorption of orally administered Cobalt-60 labelled vitamin B 12, in three of our patients with diabetic steatorrhea, also suggested intestinal alteration in accordance to the decreased urinary radioactivity levels, as previously described in patients with Idiopathic steatorrhea (13).

AKTAN and KLOTZ (16) reported that fat absorption determined by I-131 triolein and chemical fat balance was normal in uncomplicated diabetic patients. In one of two patients

with nocturnal diarrhea, described by these AA., a defect in fat absorption without definite evidence of diabetic neuropathy was disclosed, similar to two patients described by BERGE et al. (1).

In relation to case T.P., here reported, the clinical features that point to the diagnosis of celiac disease (sprue) as opposed to diabetic steatorrhea are are the following. First, the onset of steatorrhea antedated the initial diagnosis of diabetes by many years. Second, the prolonged course of the diarrhea, with remissions and exacerbations. Third, lack of evidence of neurologic complications and, finally, evidence of a nutritional deficiency state. The diagnosis of celiac disease was supported by the result of peroral biopsy of the small intestine. Unfortunately, it was not possible to verify the clinical response to steroid therapy and the result of the gluten-free-diet which the patient did not tolerate.

The abnormal xylose absorption test and the electrolyte composition of sweat ruled out the diagnosis of cystic fibrosis of pancreas.

THOMPSON (17) suggested a possible association between diabetes mellitus and celiac disease, as indicated by the familiar history of diabetes in 35% of 104 celiacs sibships as compared to 38.6% in the group of diabetic patients and only 18.4% of the group of non-diabetics with one or more diabetic relatives. Besides, this author (17) observed that the incidence of diabetes in celiac patients is significantly greater than in the general population of comparable age.

SUMMARY AND CONCLUSIONS

Nine cases of steatorrhea complicating diabetes mellitus are reported. Five of the patients showed peripheral and autonomic neuropathy, and three had chronic relapsing pancreatitis and one diabetes mellitus coexisting with celiac disease.

The pancreatic exocrine function studied through secretin stimulation alone or associated with pancreozymin was normal in the patients with steatorrhea associated with primary diabetes mellitus.

Intestinal fat absortpion was determined by the fecal excretion of I-131-labelled fats and chemical fat balance, showing abnormal absorption of the tryglyceride triolein in both primary diabetics and pancreatic disease patients when the absorption of oleic acid was normal in the latter. The patients with primary diabetes and steatorrhea also demonstrated diminished intestinal absorption of labelled vitamin B 12. In the patients with idiopathic diabetes mellitus and chronic pancreatitis where peroral jejunal biopsy was obtained no histologic changes were observed. In the case of celiac disease the jejunal biopsy was characteristic.

Our material allows clear differentiation of the cause of steatorrhea when associated with diabetes mellitus. The possible etiologic role of autonomic neuropathy involving the gastrointestinal tract has been suggested by all data in the cases where chronic pancreatic disease or sprue coul be ruled out.

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DIABETES MELLITUS GRAVE COM REMISSÃO (*)

RELATO DE UM CASO

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A acidose diabética é, geralmente, considerada conseqüência de aguda e grave insuficiência insular. Indivíduos recuperados desta complicação metabólica do diabetes, quase invariàvelmente, necessitam tomar injeções de insulina para o resto de suas vidas.

O paciente, cuja história será por nós relatada, é um caso fora do comum, visto que, após grave cetoacidose diabética, apresentou acentuada melhora, achando-se, no momento, em bom estado de saúde, normoglicêmico e aglicosúrico, sem administração de insulina e em uso de dieta livre. Trata-se de um caso de episódio diabético agudo, em adulto jovem, em que após diagnóstico precoce e tratamento adequado seguiu-se remissão do distúrbio metabólico.

O relato de "cura" de diabetes mellitus com acidose e coma constitui raridade, em que a "cura" assinalada é duvidosa e discutível. Em recente trablho, BARR (1) relata um caso de episódio diabético agudo, com remissão, em adulto de

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39 anos. Êste constituiria segundo citado autor, o sexto caso na literatura médica de remissão de diabetes, após episódio de cetoacidose.

Não é nosso propósito, na publicação dêste caso, esgotar as referências bibliográficas. Analisaremos, em particular, o conceito de "cura" do diabetes, salientando a necessidade imperiosa do diagnóstico e tratamento precoce.

APRESENTAÇÃO DO CASO

D.G.L., 26 anos, masculino, branco, solteiro, colombiano, estudante de Medicina. Procurou-nos em julho de 1962, contando uma história de 20 dias, quando começou a notar acentuada astenia, polidipsia, poliúria, emagrecimento (seu pêso caiu de 73 para 68 ks em 20 dias). Concomitantemente, notou acentuada xerose da mucosa bucal e diminuição da acuidade visual. Cinco dias antes de vir à consulta passou a sentir impossibilidade de se concentrar nos estudos, nervosismo e "hálito doce". Nasceu de parto normal, a têrmo, com pêso aproximado de 3 kg. Seus hábitos alimentares não revelaram excesso de ingestão de hidratos de carbono. Negava etilismo e tabagismo. Na anamnese familiar não relatava a existência de qualquer colateral diabético. Nada foi verificado digno de registro nos antecedentes mórbidos pregressos.

Ao exame, encontramos um paciente longilíneo, com 1,78 m e 69 kg. aparentando estar agudamente doente. Além de ter movimentos respiratórios aumentados em freqüência e intensidade, mostrava forte hálito cetônico; pele sêca, com elasticidade diminuída; ausência de xantocromia, xantelasma, necrobiose lipídica ou piodermites; mucosas ligeiramente descoradas. Pressão arterial 140 x 100 mm Hg. e pulso de 104 batimentos por minuto. E.C.G. normal. Não apresentava qualquer alteração para o lado dos aparelhos respiratório e digestivo. Ausência de alterações neurológicas; fundoscopia normal. O exame de urina revelou glicosúria e cetonúria de 4+; ausência de albuminúria. Sedimento corado normal. A glicemia era de 227 mg% com reserva alcalina de 19 mEq/1.

Aconselhado a internar-se, a fim de seguir o esquema por nós usado no tratamento da cetoacidose (dieta de 2.000 cal. dividida em 4 refeições, cada uma com 50 g de hidrato de carbono, precedida da administração de insulina regular, hidratação etc.) negou-se. Por esta razão e levando-se em conta tratar-se de estudante do 6.º ano de Medicina, que poderia acompanhar a evolução da sua enfermidade, e que estava em permanente contato conosco, concordamos em tratá-lo em sua residência. Foi prescrito dieta de 2.000 cal.,

com 200 gr de hidrato de carbono, 93,3 g de lipídios e 90 gr de proteínas, insulina lenta U-40, na dose de 40 unidades em jejum.

Decorridos 5 dias, o paciente apresntou acentuada melhora subjetiva, assinalando nítida recuperação da acuidade visual e fôrça muscular, com desaparecimento da poliúria e polidipsia. A pressão arterial foi de 130 x 80 mm de Hg.; a glicemia baixou para 190 mg% com completo desaparecimento da cetonúria, bem como do hálito cetônico; a glicosúria na urina das 24 horas era de 15 gr. Diminuimos a dose diária de insulina para 24 unidades. Uma semana após, o paciente se apresentava com glicemia de 150 mg%, aglicosúrico, bem disposto, tendo retornado aos estudos e ao trabalho.

Três semanas após, retornou à consulta, referindo progressiva melhora, e apresentando-se normoglicêmico (118 mg%) e aglicosúrico. Diminuimos, então, a insulina para 16 unidades e administramos tolbutamida na dose de 3 g no primeiro dia, 2.5 g no segundo e 2 g no terceiro, 1,5 g no quarto e estabelecemos 1 g como dose de manutenção.

Uma semana após, já com dois meses de tratamento, como o paciente se apresentava assintomático, com glicemia de 120 mg%, aglicosúrico, diminuimos ainda mais a insulina para 12 unidades e mantivemos a administração de 1 g diário de tolbutamida.

Decorridos 15 dias, a glicemia era de 90 mg%; retiramos a insulina, continuando sòmente com a dieta e tolbutamida. Voltou a nos procurar, uma semana após, com glicemia de 80 mg%, inteiramente assintomático, motivo pelo qual reduzimos a dose de tolbutamida para 0,5 gr e, finalmente, uma semana após, retirou-se êsse medicamento, continuando o paciente em uso exclusivo de dieta. Gradativamente, foi essa liberada. Sua glicemia tem-se mantido em tôrno de 100 mg%, permanecendo inteiramente assintomático há 2 anos.

DISCUSSÃO

Acentuada melhora do estado diabético em conseqüência de tratamento, foi pela primeira vez salientada pelo médico inglês JOHN ROLLO (2), em 1796. No século seguinte, o tratamento dietético do diabetes — restrição acentuada de hidratos de carbono, bem como de proteínas e gorduras — sistematizado por CANTANI, BOUCHARDAT e NAUNYN, segundo relata ALLEN (3) no seu trabalho sôbre a história do tratamento do diabetes sacarino, levou êsses autores clássicos a registrar numerosos casos de diabetes, onde o uso de dieta acentuadamente hipocalórica gradualmente melhorava a tolerância aos hidratos de

carbono, a ponto de se tornarem os pacientes normoglicêmicos e aglicosúricos por muitos anos, mesmo após volta ao uso de dieta normal.

ALLEN (4), revendo a literatura acêrca da curabilidade do diabetes, cita a opinião de NAUNYN: "a "cura" do diabetes talvez exista, porém constitui raridade, não conhecendo eu nenhum caso em que tenha ocorrido após duração prolongada da enfermidade". O mesmo autor dá ênfase à distinção entre "melhora" e "cura" do diabetes, salientando a necessidade de muito cuidado em se empregar o têrmo "cura", devido ao caráter intermitente de alguns casos. "Diabético curado", escreve o citado autor, "é um indivíduo que pode viver como tôdas as outras pessoas, em uso de alimentação habitual, com quantidades normais de hidratos de carbono, permanecendo livre de sintomas e sinais de enfermidade, normoglicêmico, aglicosúrico... Usando êste critério, numerosos casos relatados de cura deixam de o ser".

NEWBURGH e CONN (5), relatam o "desaparecimento" do diabetes em pacientes obesos, após a normalização de seu pêso. Ditos pacientes tornam-se assintomáticos e aglicosúricos; com a regularização do pêso, a curva de tolerância aos hidratos de carbono se normalizava.

Alguns dêstes enfermos, abandonando a dieta e tornando a engordar, novamente apresentavam diminuição de tolerância aos hidratos de carbono e sintomatologia diabética. Em trabalho posterior, NEWBURGH (6), motifica a noção por êle defendida anteriormente, mostrando que os seus diabéticos obesos, tornados normoglicêmicos e assintomáticos, apresentavam curvas glicêmicas do tipo francamente diabético.

Numerosos autores relatam o "desaparecimento" da enfermidade em raros casos de forma infanto-juvenis (7, 8, 9 e 10).

Estudos sôbre a patologia do diabetes e sua produção em animais de laboratório, vieram trazer alguma luz para a melhor compreensão da eventual "remissão" dêste distúrbio metabólico. MAC CALLUM (11), descrevendo achados de autópsia em duas crianças, que faleceram em quadro diabético agudo, notou alterações no pâncreas, por êle interpretadas como hipertrofia compensadora e regeneração das Ilhotas de Langerhans. Esta

regeneração insular se fazia à custa do epitélio dos ductos acinosos. CECIL (12) relata sinais histopatológicos de hipertrofia e neoformação de tecido insular em 34 das 100 autópsias de pacientes diabéticos. WARREN e ROOT (13) escreveram: "os achados patológicos por nós encontrados no pâncreas nas autópsias de diabéticos, raramente representam o dano inicial do órgão, mas sim o resultado de processos regenerativos do tecido insular, bem como alterações degenerativas causadas pelo fator diabetogênico. O pâncreas não é um órgão estático como o cérebro ou o miocárdio, incapazes de apresentarem sinais de regeneração após a injúria". GRAY e FEEMSTER (14), notaram acentuada hipertrofia e hiperplasia das Ilhotas de Langerhans em recém-nascidos de mães diabéticas, mostrando, portanto, a capacidade de hiperplasia compensadora do pâncreas endócrino.

ALLEN (15), praticando pancreatectomia subtotal em cães, encontrou resultados que nos parecem de grande importância. Assim, relata o autor, que o tipo de dieta usado após a operação tem capital importância no quadro clínico desencadeado pela experiência, bem como nos achados histológicos no tecido pancreático remanescente. Quando se dava aos animais dieta hipercalórica e rica em hidratos de carbono, apresentavam êles grande perda de pêso, cetoacidose grave, acentuada degeneração hidrópica das ilhotas. Já os animais em que após a operação se administrava dieta com restrição de glicídios, gradualmente ganhavam pêso, aumentava a tolerância dos hidratos de carbono e na autópsia era encontrada hipertrofia e hiperplasia das ilhotas. COPP e BARCLAY (16) mostraram que a administração de insulina após pancreatectomia subtotal em cães igualmente previne a degeneração hidrópica das ilhotas remanescentes, tendendo a promover a hiperplasia do tecido beta insular.

HAIST, CAMPBELL e BERT (17), estudando a produção experimental de diabetes, concluem que em animais onde se faz pancreatectomia subtotal ampla, a administração de hipófise anterior leva à exaustão funcional e degeneração das ilhotas remanescentes, com grande diminuição da insulina pancreática e grave diabete clínico. Se nos mesmos animais se administra

dieta pobre em hidratos de carbono, bem como insulina, a degenreação insular não se fará, não aparecendo o quadro clínico do diabetes. DOHAN e LUCKENS (18), produziram diabetes em gatos com injeções intraperitoniais continuadas de glicose. Concluem que a hiperglicemia induzida por êste processo é o fator causal de dano insular, podendo levar a franco estado diabético.

À luz dêstes dados experimentais, os relatos clínicos de remissão em diabetes humano podem ser melhor compreendidos.

BOYD e ROBINSON (19) relatam o caso de uma criança diabética de 9 anos, onde a administração inicial de insulina era de 90 unidades. Gradualmente, as necessidades de insulina diminuiram, chegando a 30 unidades diárias. Aos 13 anos, achando-se a paciente em bom estado geral, compensada do seu distúrbio metabólico, sofreu acidente de tráfego, vindo a falecer. A necrópsia revelou acentuada hiperplasia e regeneração das Ilhotas de Langerhans.

LEYTON (20), discutindo a possibilidade de "recuperação" em diabete sacarino afirma: "a maioria dos autores aconselha a administração de mínima dose de insulina, de modo a tornar o paciente aglicosúrico em dieta balanceada, hipoglicídica; eu administro doses bem maiores de insulina, de modo a torná-lo não sòmente aglicosúrico, mas sim normoglicêmico". São descritos pelo autor 9 pacientes com "recuperação" do diabetes onde as doses de insulina foram gradualmente reduzidas e, finalmente abolidas, devido ao aparecimento de sinais hipoglicêmicos. Êsses pacientes se mantiveram em dieta com restrição de glicídios de 6 meses a 2 anos. A análise minuciosa de todos êsses casos mostra que havia simplesmente "remissão" e não "cura", pôsto que, todos êles apresentavam hiperglicemia após infecções ou abusos alimentares. Assinala o autor, que a incapacidade em produzir similar remissão em outros pacientes diabéticos, pode ser atribuída a vários fatôres:

1 — Retardo no tratamento.

2 — Infecções de repetição.

3 — Falta de cooperação do paciente ou mesmo dificuldades técnicas em manter a glicemia abaixo de 130 mg%, especialmente à noite.

Nos últimos 20 anos, numerosos trabalhos têm aparecido sôbre possibilidade de se conseguir acentuada melhora de estados diabéticos graves, desde que tratados intensa e precocemente. Assim, JACKSON, BOYD e SMITH (21), com grande experiência no tratamento do diabete infantil, assinalam que freqüentemente as necessidades diárias de insulina são reduzidas para metade ou 1/3, desde que o tratamento seja iniciado precocemente. Êsses autores dão ênfase ao fato de que, comumente, os médicos, para evitar acidentes hipoglicêmicos, têm tendência em manter o doente com pequena glicosúria e, portanto, com hiperglicemia crônica, tão danosa para o tecido insular. BRUSH (22), informa experiência similar, registrando que em 39 pacientes com diabete juvenil o uso de doses adequadas de insulina, a ponto de torná-los ràpidamente normoglicêmicos, melhorava acentuadamente sua tolerância aos hidratos de carbono, sendo necessário, por vêzes, reduzir a dose diária de insulina para menos de 10 unidades. Já outros autores, que têm receio de administrar doses suficientes de insulina, necessitam, para o contrôle, doses bem maiores, em tôrno de 30 unidades diárias.

LUKENS e DOHAN (23) descrevem os casos de 19 pacientes com diabete infantil que, com a continuação do tratamento, apresentavam acentuada melhora, mantendo-se normoglicêmicos e aglicosúricos, sòmente com dieta. Apesar da normalização do quadro clínico e laboratorial, a curva glicêmica sempre se mostrou francamente diabética.

HARTMAN (24) relata duas observações clínicas de diabete infantil, de início agudo, levando à acidose e coma. Após o tratamento adequado, permaneceram normoglicêmicos por vários meses, simplesmente com a dieta. Assinala o autor que vários fatôres podem contribuir para desencadear de maneira súbita a cetoacidose: estados infecciosos, excesso de ingestão de hidratos de carbono, crises emocionais e superprodução de antagonistas da insulina.

CHENG, JAHRANS e TRAUT (25) referem o caso de uma senhora de 62 anos que deu entrada no serviço com cetoacidose, glicemia de 1.120 mg% e reserva alcalina de 10 vol. de CO^2. Foram necessários 1.425 unidades de insulina, nas primeiras

24 horas, para retirá-la do coma. Subseqüentemente, o seu estado clínico foi progressivamente melhorando, com diminuição das necessidades diárias de insulina, tornando-se normoglicêmica e aglicosúrica. Sua curva glicêmica, no entanto, se apresentava com nítida diminuição de tolerância. Outro caso, clássico na literatura, é o de GRECO e SCAPELLATO (26). Tratava-se de um homem de 62 anos que, durante a convalescença de pneumopatia aguda, consumiu dois quilos de açúcar e três quilos de mel em dois dias. Desenvolveu-se, ràpidamente, grave quadro diabético com cetoacidose; a glicemia chegou a 640 mg% e a reserva era de 18 vol. de CO^2. Após tratamento com insulina e hidratação, houve grande melhora do quadro metabólico. Gradativamente, a insulina foi diminuída, a ponto de ser retirada. Dois anos após, o paciente encontrava-se em dieta livre, assintomático, normoglicêmico e aglicosúrico e com curva glicêmica normal.

BARR (1), relata um caso de remissão de diabetes em homem de 39 anos, após episódio agudo de cetoacidose e coma. O doente respondeu bem ao tratamento do coma, continuando a terapêutica insulínica, por várias semanas; com o passar do tempo surgiram sintomas hipoglicêmicos, obrigando à diminuição progressiva da insulina diária. Permaneceu normoglicêmico e aglicosúrico 24 meses, em dieta livre, sem uso de insulina.

Desta revisão bibliográfica conclui-se que acentuada melhora pode ocorrer em diabéticos do tipo lipopletórico, com início da enfermidade após os 40 anos, simplesmente pela restrição dietética, com redução de pêso. Igualmente, pode haver remissão, em raríssimos casos do tipo juvenil com grande carência de insulina endógena, quando tratados de maneira precoce e intensa. As noções atuais sôbre a patologia do diabetes em animais de laboratório podem dar explicação conveniente para estas remissões: as células beta das ilhotas, em diversas circunstâncias, que determinam hiperglicemia, podem sofrer grande estímulo, a ponto de haver exaustão funcional. Se o estímulo hiperglicemiante é ràpidamente removido, seja pela restrição de hidratos de carbono, administração de insulina, normalização de um hipercorticismo, retirada de feocromocitoma

ou a cura de uma tireotoxicose, poderá haver recuperação do tecido exaurido, com normalização da produção de insulina, levando à normalização da tolerância aos glicídios.

Uma pergunta tem pleno cabimento: porque esta remissão não é mais freqüente? Várias são as razões que poderíamos alinhar:

1.°) — O diabetes é uma doença hereditária, genética, resultante de mutação punctiforme e, em conseqüência, por definição, doença incurável.

2.°) — O paciente só procura o médico após vários meses ou mesmo anos de duração da enfermidade. Nesse período a hiperglicemia já condicionou exaustão do tecido beta insular, hereditàriamente lesado.

3.°) — Todos os especialistas não ignoram a dificuldade em se conseguir que o doente faça dieta. Assim, é comum, indivíduos em tratamento ambulatorial necesistarem doses de insulina bem maiores do que quando internados.

4.°) — Com freqüência, um diabético, por má orientação do médico, não atualizado em assuntos de nutrição, leva muito tempo para normalizar a glicemia, seja pelo uso de dieta inadequada ou seja por dose insuficiente de sulfoniluréia ou insulina. A hiperglicemia decorrente desta má orientação conduzirá à exaustão pancreática definitiva.

5.°) — Os médicos, freqüentemente, receiosos de acidentes hipoglicêmicos, preferem manter seu paciente com moderada hiperglicemia e pequena glicosúria.

6.°) — A maioria dos pacientes, que já sofreu qualquer acidente hipoglicêmico, com mêdo de repetição, prefere comer um pouco mais do que o prescrito ou diminuir a dose recomendada de insulina.

A recuperação de um doente diabético, após um episódio de cetoacidose, constitui raridade. Isto é fàcilmente compreensível: um estado diabético grave, que conduziu à cetoacidose, indica ausência de insulina endógena por lesão do tecido insular. O caso por nós descrito torna-se mais interessante por não encontrarmos nos seus pródromos qualquer herança diabética, bem como fatôres desencadeantes (estados infecciosos "stress" emocional, intervenções cirúrgicas, excesso de ingestão

de glicídios, etc.). Também não foi constatado quadro de pancreatite aguda, assinalada por TULLY e LOWENTHAL (27), como fator desencadeante de cetoacidose de início agudo. Outro aspecto que merece destaque, é o fato de ter o paciente entrado em cetoacidose grave com glicemia de sòmente 227 mg%.

Acha-se êle, no momento, normoglicêmico e aglicosúrico há 24 meses, em uso de dieta livre. Acreditamos não se tratar de um caso de 'cura". Uma observação mais longa será necessária a fim de confirmar a remissão. Neste caso, apesar de se tratar de um caso de diabete juvenil, onde está formalmente contra-indicado o uso de sulfoniluréia, foi ela administrada com a finalidade de condicionar a neoformação das células beta. Essa neoformação, negada por todos os autores é, no entanto, referida por LOUBATIÉRES (28) num caso de certa menina de 14 anos, com diabete recente, onde o tratamento dietético com sulfoniluréia normalizou o quadro clínico e laboratorial, permanecendo assintomática durante 3 anos.

CONCLUSÕES

O têrmo "cura" do diabetes deve ser usado excepcionalmente, com extrema reserva, e sujeito a severas críticas. O ditado "uma vez diabético, sempre diabético" é aceito por unanimidade. Revendo a bibliografia no assunto, BARR (1), em 1961 declara que, em realidade, só encontrou 5 casos de cura com "follow-up" longo. Nesses casos, os pacientes não sòmente tornaram-se assintomáticos, normoglicêmicos e aglicosúricos, como as curvas de tolerância aos hidratos de carbono retornaram à normalidade, atestando a recuperação funcional do pâncreas endócrino. Êsses casos seriam: HARDWOOD (29); JOHNSON (30) com dois casos; CHENG e cols. (25) e DEL GRECO e SCAPELLATO (26). Outros casos relatados na literatura, segundo o mesmo autor, não resistem a acurado exame, havendo falhas nítidas na observação clínica e laboratorial, pôsto que, todos êles submetidos a curvas glicêmicas, estas se mostravam com nítida diminuição de tolerância.

Ignoramos se o nosso paciente, no futuro, submetido a 'stress" emocional, estados infecciosos ou sobrecarga alimentar

não apresentará gradativa e progressivamente, deficit do pâncreas endócrino com aparecimento dos clássicos sintomas do diabete sacarino. Acreditamos que o diagnóstico precoce com tratamento intenso e adequado, bem como a colaboração do paciente, muito contribuiram para o êxito por nós obtido, abrindo novas perspectivas para que se tente obter a "remissão" em diabéticos juvenis uma vez tratados logo de início.

RESUMO

Os autores registram um caso de diabetes em adulto jovem com início súbito, levando a grave cetoacidose, não tendo sido registrado nos pródromos, qualquer herança diabética bem como fatôres desencadeantes. Após tratamento com dieta e insulina, houve desaparecimento da sintomatologia clínica e normalização laboratorial. Na fase final do tratamento, foi administrado tolbutamida. Gradativamente foi liberada a dieta, achando-se o paciente há 24 meses, normoglicêmico, aglicosúrico, sem uso de insulina ou sulfoniluréia, em dieta livre.

Após rever na literatura o conceito de remissão de diabetes sacarino, os autores concluem que o têrmo "cura" do diabetes deve ser usado excepcionalmente, com extrema reserva e sujeito a severas críticas. Assim, creem ser possível, que no caso por êles registrado, no futuro, quando submetido a "Stress" emocional, estados infecciosos ou sobrecarga alimentar, surgirá gradativa e progressivamente, insuficiência pancreática endócrina com aparecimento dos clássicos sintomas do diabetes.

Os autores acreditam que o diagnóstico precoce com tratamento intenso e adequado muito contribuiu para o êxito por êles obtido.

SUMMARY

A case presented of diabetes in a young adult, with a sudden onset to which no contributing factors were associated, leading to severe Kitoacidosis. There were neither prodromic manifestations nor diabetic traits in the family. After insulin and diet treatment symptoms disappeared an laboratory tests became normal. During the latter stage of treatment tolbutamide was administered. The diet was progressively liberalized until it became

free. The patient's blood sugar is now normal and his urine is sugar-free.

A careful revision of the subject reveals that the expression "cure" related to diabetes mellitus has always been, and should continue to be, used only with extreme care, as one open to criticism. It is believed that the present case may show pancreatic insufficiency and diabetic symptoms in the future, if and when the patient is subjected to emotional stress, infection or overeating. Anyhow, an early and adequate treatment certainly explains the present therapeutic success.

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O ÍNDICE CLÍNICO DE WAYNE NO DIAGNÓSTICO DO HIPERTIREOIDISMO

SANTOS MOURA (**), FERNANDO ALMEIDA (***) e FRANCINEIDE NASCIMENTO (****)

INTRODUÇÃO

Embora os atuais meios semiológicos PBI e Radiodo permitam realizar o diagnóstico do hipertireoidismo com grande precisão, limitados que são aos grandes centros, nem sempre é dado ao médico dispor dos mesmos, obrigando-o, assim, a valer-se de meios auxiliares laboratoriais menos precisos e sobretudo, dos dados clínicos mais sugestivos que, convenientemente avaliados podem constituir subsídios definitivos para feitura diagnóstica.

WAYNE (1), realizando uma análise estatística da sintomatologia do hipertireoidismo atribui um determinado valor numérico, positivo ou negativo, aos sinais e sintomas cuja presença ou ausência se revelaram importantes para estabelecer o diagnóstico (tabela 1).

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Êsse método passou a ser conhecido como Índice Diagnóstico Clínico — I.D.C. — (Clinical Diagnosis Index).

O referido autor analisou, inicialmente, um grupo de pacientes com hipertireoidismo, comparando-o com um grupo de pessoas normais ou portadoras de tireoideopatias sem o componente de hiperfunção. Pôde assim concluir que, através da soma algébrica dos valores atribuídos aos sinais e sintomas,

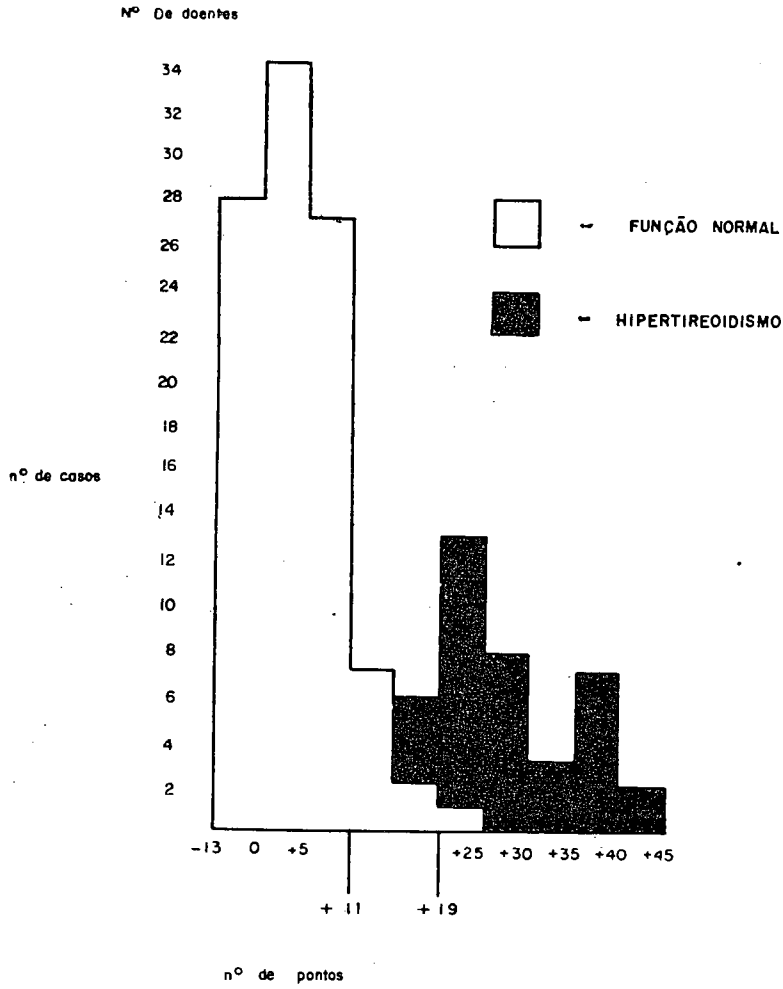

Fig. 1 — Distribuição de 135 pacientes de Tireoidiopatias de acôrdo com o teste de Wayne

os hipertireoidianos apresentaram um valor nunca inferior a 19 pontos, ao passo que os normais não ultrapassaram valores acima de 11 pontos positivos. Os valores intermediários, isto é, entre 11 e 19 pontos, passaram a constituir uma faixa de diagnóstico duvidoso.

Aplicando o I.D.C. a um grupo de pacientes sem diagnóstico prévio obteve o autor uma alta percentagem de elucidação diagnóstica (88%), devendo ressaltar-se que, posteriormente, todos os casos tiveram a confirmação diagnóstica não sòmente laboratorial como também o sucesso terapêutico.

É nosso intuito neste trabalho, tendo em vista a simplicidade do teste e sua fácil execução, verificar as possibilidades de sua aplicação em nosso meio, obedecendo ao critério original do autor.

MATERIAL E MÉTODO

Estudamos 135 pacientes portadores de tireoideopatias, atendidos no Setor de Endocrinologia da 1.ª Cadeira de Clínica Médica, no período de 1957 a 1962. Excluimos as observações que, incompletas ou inadequadas, não permitiram uma aplicação correta do Índice.

Todos os nossos casos tiveram os seus diagnósticos confirmados com o auxílio de um ou vários exames especializados (Metabolismo basal P.B.I. — 127 e Radioiodo), ao lado da respectiva comprovação terapêutica e, em alguns casos, exame anátomo-patológico, sendo a seguinte a sua distribuição de acôrdo com o diagnóstico:

Bócio Difuso Atóxico	53
Bócio Difuso Tóxico	31
Bócio Nodular Atóxico	43
Bócio Nodular Tóxico	7
Câncer	1

RESULTADOS

Com a aplicação do I.D.C. em nossos pacientes obtivemos confirmação diagnóstica total em 89,6% dos casos (fig. 1).

No grupo de pacientes com hipertireoidismo o teste re-

velou-se positivo em 89,5% dos casos, enquanto que no grupo normofuncionante a concordância diagnóstica foi de 89,7%.

ÍNDICE DIAGNÓSTICO DE WAYNE

Sintomas

1 — Preferência para o frio	+ 5
2 — Sudorese abundante	+ 3
3 — Aumento do apetite	+ 3
4 — Diminuição do pêso	+ 3
5 — Palpitações	+ 2
6 — Fraqueza	+ 2
7 — Nervosismo	+ 2
8 — Dispnéia de esfôrço	+ 1
9 — Indiferença à temperatura	0
10 — Diminuição do apetite	— 3
11 — Aumento do pêso	— 3
12 — Preferência para o calor	— 5

Sinais	presente	ausente
1 — Movimentos hipercinéticos	+ 4	— 2
2 — Fibrilação auricular	+ 4	
3 — Tireóide palpável	+ 3	— 3
4 — Pulso acima de 90 b.p.m.	+ 3	
5 — Frêmito na tireóide	+ 2	— 2
6 — Exoftalmia	+ 2	
7 — Retração palpebral	+ 2	
8 — Mãos quentes	+ 2	— 2
9 — Assinergia óculo-palpebral	+ 1	
10 — Tremor das extremidades	+ 1	
11 — Mãos úmidas	+ 1	— 1
12 — Pulso entre 80 e 90 b.p.m.	0	
13 — Pulso menor que 80 b.p.m.	— 3	

+ 11 + 19

faixa de dúvida

eutireoideos hipertireoideos

COMENTÁRIOS E CONCLUSÕES

Analisando os resultados obtidos observamos que o I.D.C. é incontestàvelmente uma contribuição valiosa para o diagnóstico clínico do hipertireoidismo, desde que o observador se capacite a uma investigação mais ampla dos sinais e sintomas apresentados pelos pacientes pois, como já acentuou o próprio WAYNE, o resultado do teste não depende de ser o mesmo realizado por um especialista. Devemos, entretanto, ressaltar a conveniência de ser o diagnóstico clínico do hipertireoidismo, sempre que possível, confirmado por um teste objetivo, antes que o tratamento seja iniciado — TROTTER (2).

A concordância diagnóstica por nós obtida — 89,5% foi bastante significativa, situando-se um pouco acima dos resultados obtidos pelo próprio autor do teste.

Não podemos deixar de salientar que no nosso material foi registrada uma alta incidência de pacientes com infestação verminótica, na maioria dos casos múltipla, associada a estados multicarenciais de evolução anterior ao aparecimento da sintomatologia sugestiva de hipertireoidismo. Aparentemente, êsses fatôres não modificaram a acuracidade do índice.

No presente momento não podemos ajuizar sôbre quais os fatôres responsáveis pela inclusão dos casos que se situaram na faixa do diagnóstico duvidoso.

RESUMO

Os autores aplicaram o índice Diagnóstico Clínico de Wayne a 135 pacientes portadores de tireoideopatias no sentido de observar sua acuracidade no diagnóstico do hipertireoidismo.

Em 89,6% dos casos houve concordância com o diagnóstico final, variando de 89,7% para os eutireoidianos e 89,5% para os hipertireoidianos.

Concluem ser na realidade um teste qualitativo de grande valor diagnóstico, ao lado da simplicidade e facilidade de execução.

SUMMARY

The authors studied the accuray of Wayne's Clinical Diagnosis Index for diagnosis of hyperthyroidism in 135 patients with thyroid diseases.

In 89,6% of the cases the test was concordant with the final diagnosis ranging from 89,5% for the patients with hyperthyroidism to 89,7% for the patients with euthyroidism.

The test is of easy execution and has been considered as highly valuable in the diagnosis of hyperthyroidism.

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METHODS IN HUMAN CYTOGENETICS (1)

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It is a surprising fact that although the chomosomes were described rather early in the history of microscopy, it is only very recently that human chromosomes have been studied extensively. In part, this was because human, and indeed all mammalian chromosomes are "dificult" cytological material. In fact, it was only as recently as 1956 that the use of tissue cultures and of special preparative techniques permitted TJIO and LEVAN (1956) to ascertain that the normal human chromosome number is actually 46, and not 48, as had long been believed. But although the development of these new techniques then permitted detailed study, another three years elapsed before work on human chromosomes became really active. The discovery by LEJEUNE, GAUTIER, and TURPIN (1959) that a human disease state, namely mongolian idiocy, or Down's syndrome, was associated with an

(1) It is with great pleasure that this paper is dedicated to Professor Sajiro Makino of the Zoological Institute of Hokkaido University, a pioneer in mammalian chromosome cytology, in honor of his 60th birthday.

(2) Operated for the U.S. Atomic Energy Commision by Union Carbide Corporation.

abnormality of the chromosomes provided the necessary stimulus. Since then extensive interest has developed, and a new discipline, generally called human cytogenetics, has been created. The nature of this new field is closely bound up with the special technical problems and methods involved.

During most of the life cycle of a cell the chromosomes cannot, of course, be seen at all. It is only when they are contracted during a cell division, either a mitosis or a meiosis, that they may be examined with the microscope, counted, and analysed. The meiotic divisions of germ line cells have been studied only rarely in human cytogenetics. Although they would be very suitable for some types of studies, the great difficulty of obtaining useful cytological preparations, as well as the problem of obtaining the gonadal tissue itself has caused most investigators to confine their work to the mitoses of somatic cells. Thus for practical purposes the object of study in human cytogenetics, unlike that of earlier work in other areas of cytogenetics, is the somatic cell. Such somatic cells are generally diploid, having two of each type of chromosome, one derived from the male and the other from the female parent. The normal human, then, is characterized by 23 pairs of chromosomes. The members of each pair are identical in the cells of normal females. In the male, however, the chromosomes of one pair, the sex or X-Y pair, are different in morphology.

Each pair of chromosomes is characterized by a particular morphology. These are not, however, necessarily unique, at least at the level of observation and measurement with the light microscope. Fig. 1 shows the chromosomes in a dividing leukocyte from a normal human male. Each chromosome is composed of two strands called chromatids, joined together at a relatively unstained region called the centromere. The chromatids are actually the identical daughter chromosomes resulting from the replication of the single chromosomes during the preceeding interphase. The region of attachment, the centromere, is also the point of attachment of the chromosomes to the cell's spindle apparatus. At the anaphase of mitosis the centromeric attachment of the two chromatids

disappears, and one of the chromatids of each pair moves to each of the poles of the spindle. When the cleavage of the cell is completed each daughter cell thus has a complete complement of chromosomes. Each is, however, now single, and must be replicated before the next mitosis. The relative lengths and centromere positions for each chromosome are constant, within the limits imposed by their elasticity, from cell to cell. Although a few species have been shown to be chromosomally polymorphic, the chromosomes of most, including man, have a characteristic number and form with only minor differences in normal individuals. These are likely to differ between species, but usually not between normal individuals within a species, nor between cells from one individual. It is the abnormal deviation of individuals or of individual cells from the normal pattern which constitutes the major subject of study in human cytogenetics.

Methods of obtaining suitable cells in mitotic division, of making well spread, fixed, and stained chromosome preparations, and of analysing these preparations constitute the methodology of human cytogenetics. There are a number of features common to almost all of the techniques in use. Almost always the cells are induced to divide *in vitro* in a tissue culture growth medium. Usually the cells are handled as suspensions of individual cells, rather than as solid tissue. The drugs colchicine or colcemid are almost universally used to arrest cells in metaphase and to facilitate spreading and flattening of the chromosomes in the final preparations. A pre-fixation treatment with hypotonic fluid is used to swell the cells and to improve spreading of the chromosomes. Finally, some method of flattening the fixed cells on the slide, such as squashing or drying, is always used. Probably because the field is a new one, virtually as many different techniques as there are investigators working in it have been described in the literature. Most differ from others only in minor details, however. Since it is neither possible nor desirable to describe all of these variations here, an example of each of the important types of method will be given. The examples selected are, with one exception (the direct bone narrow

method of BOTTURA and FERRARI, 1960), those I use myself. I must hasten to add that this should in no way be taken to mean that these are the only techniques which will work, or even that they are the most suitable ones for others to use. They are, however, simple and they produce reasonable results. In any case, minor differences from one investigator's technique to another's rarely produce major changes in the results; more frequently than not they are simply matters of preference or of convenience. Thus the reader should not hesitate to adapt the following methods, or others, to his own personal requirements.

CELL PREPARATION

A. *General considerations.*

Most cell preparation methods require the maintenance of the cells *in vitro* for at least a few hours. They are all, in a sense, tissue culture methods. Many of the general requirements for mammalian tissue culture are thus applicable; many failures of cytogenetic methods may be traced to a failure to meet them. Fortunately sterility, long a particular problem in mammalian tissue culture work has become much less of a worry since the advent of antibiotics. Penicillin and streptomycin are usually added to culture media so that only resistant strains (unfortunately common around hospitals now) and various fungi present a problem. For these, ordinary bacteriological sterile technique generally suffices. In any case, since the human cytogeneticist's cultures are usually short term ones, the loss of an occasional contaminated culture is tolerable. Other problems can be more general, and thus more serious.

1. *Glassware.* Tissue cultures, especially those in which the cells are attached to the glass surface itself, are very sensitive to their containers. Some types of glass are actually toxic to mammalian cells. In general, borosilicate glass is quite safe. Many soft glasses are also acceptable, but it is best to test new items before putting them into service. Plastic

"throw away" culture containers are available, but many types are quite toxic to mammalian cells, and only those manufactured specifically for tissue culture should be used.

Washing of glassware can also be critical. Many soaps and detergents commonly used in the laboratory are toxic, and some are remarkably difficult to rinse off the glassware once they have been used. The same applies to acid cleaning solutions, particularly if technical or industrial grade chemicals are used in their preparation. In general it is best to use one of the special non-toxic cleaners sold especially for tissue culture glassware. The water used to wash and rinse glassware may also contain toxic materials. Some may contain materials which will make the surface of the glass non-wettable, a condition which may be fatal to cultures of cells which must attach to the glass. Silicone greases, anti-foam agents, and even handcreams are particular hazards, as they are frequently almost impossible to remove. Sterilization of glassware in steam containing even traces of such materials, or even in autoclaves or ovens which have recently contained such materials, has occasionally been responsible for culture failures.

2. *Rubber and metal items.* Rubber or metal items which come into contact with media or cells must also, of course, be non-toxic. These may include hypodermic needles, possible, tubing, filters, and stoppers. It is worth while, when possible, to use only items which are safe for parenteral use. In general, stainless steel, and special white and silicone rubber formulations are safe, as are most amber pure gum rubbers. Such items should also be washed carefully, of course. It is worth noting that some items, such as rubber stoppers, even though they may not come into actual contact with the medium, may contain volatile toxic materials and thus cause culture failure.

3. *Media.* Naturally, only pure reagents should be used in the preparation of media and solutions. Impure grades of some chemicals contain toxic amounts of heavy metal ions. Water used for media should be distilled, and suitable for parenteral use. Media must, naturally, be sterilized; frequently filtration is the only acceptable method. Attention should be given to the filters themselves, as some types, such as

asbestos pads, can themselves introduce toxic materials into the medium they are used to filter. Since the preparation of many of the media used for tissue culture is very tedious and complex, and since only small amounts are usually required by a cytogenetics laboratory, it is generally desirable, where feasible, to purchase them ready-made from a commercial supplier. Several suppliers make up special units for this type of use, which may be stored conveniently for long periods.

4. *pH control.* Mammalian cells are, of course, quite sensitive to pH. When they are separated from the normal homeostatic mechanisms of the body, they are at the mercy of the culture medium. Virtually all tissue culture media are buffered with bicarbonate. In consequence, the pH of the culture depends not only on the medium itself, but on an equilibrium between the medium and the partial pressure of CO_2 in the atmosphere above the liquid phase in the culture vessel. If CO_2 can diffuse out of the culture container, the medium will become quite alkaline, due to the continous loss of CO_2 from the liquid phase. For most media, equilibrium with an atmosphere containing 5% CO_2 will yield a pH near neutrality. The simplest control method is to make the cultures in gas-tight containers, using screw caps with rubber liners, or rubber stoppers to effect a seal. In some culture laboratories, a controlled CO_2 atmosphere is provided inside an incubator; in this case, containers can be left unsealed to take advantage of the somewhat better buffering capacity such a control system provides.

This list of requirements and considerations for tissue culture work may seem somewhat formidable. It should not, however, be allowed to discourage the potential cytogeneticist. Once a satisfactory system has been worked out, the simple expedient of not changing *anything* unless it becomes absolutely necessary will serve to prevent difficulty. If changes do become necessary, only one change should be made at a time, so that trouble, if any, can be traced directly to the new material or technique. Should problems be encountered at the very beginning, it is usually possible to seek the help of someone already making satisfactory cultures. Substituting

the doubtful new items one at a time into the routine which is already giving satisfactory results should identify the cause of the trouble in a very short time.

B. *The "direct" bone marrow method.*

One of the obvious requirements if a tissue is to be suitable for *direct* cytogenetic analysis is that it must undergo active cell division *in vivo*. Another is that it must be readily accessible. Bone marrow meets both of these requirements. This tissue has the further advantage that single cell suspensions may be prepared easily. The method used for human marrow is an adaptation of one developed by FORD and HAMERTON (1956). It may be noted that the method is also suitable for other tissues, provided only that they may be reduced to a suspension of cells by some process, such as aspiration through the narrow orifice of a hypodermic needle, or by enzymatic digestion.

1. Marrow is aspirated from the patient, usually from the sternum, by means of one of the standard instruments. Two to five millititers of blood and marrow are placed in ten millititers of medium TC199 (MORGAN, MORTON, and PARKER, 1950) supplemented with 10% human or calf serum, in a suitable small bottle or flask. Colchicine is added, generally from a concentrated stock solution, to a final concentration of 10^{-4} *M*. The suspension is repeatedly aspirated with a hypodermic syringe and needle in order to produce a uniform cell suspension, as free as possible from cell clumps. The culture is then incubated at 37°C for three to five hours. Since the culture is to be fixed only a few hours after it is made, it is not necessary to observe the usual precautions about sterility.

2. After the required incubation time has elapsed, the culture is treated, fixed, and mounted by the method described below for preipheral leukocyte cultures.

3. BOTTURA and FERRARI (1960) have injected the relatively non-toxic colchicine derivative Colcemid (CIBA) directly into patients in order to collect metaphases, thus eliminating the

need for an *in vitro* culture period. They used a dose of 0.1 mg/kg body weight, injected intravenously, and aspirated bone marrow two hours later. This is, of course, a *medical* procedure, and its advisability must in each case be judged by the physician responsible for the patient. BOTTURA prepared the cells by the Feulgen-squash technique of FORD and HAMERTON (1956), but there seems to be no reason why the technique described below for peripheral leukocytes might not also be used for bone marrow cells from Colcemid-treated patients.

C. *The peripheral leukocyte technique.*

By far the most widely used technique at present is the peripheral leukocyte technique described by MOORHEAD *et al.* (1960). Perhaps the major reason for the technique's popularity is the ease of obtaining the required tissue specimens. Curiously, the success of this technique depnds on the accidentally discovered ability of the substance phytohaemagglutinin to stimulate mitosis in peripheral lymphocytes. Phytohaemagglutinin is a rather crude aqueous extract from any one of a variety of different species of beans. It is a powerful non-specific blood cell agglutinin. It was, in fact, the use of the agglutinating property of this material for the separation of white cells for culture which led to the discovery that it also had a powerful mitogenic effect on certain white blood cells.

1. A sterile five milliliter peripheral blood specimen is obtained by venipuncture, using a syringe wet with 0.1 ml of 1000 unit/ml heparin. Alternatively, if other procedures require non-heparinized blood, the blood may be collected with a dry syringe and a five milliliter sample immediately placed in a sterile tube containing the heparin. The heparinized blood sample is placed in a centrifuge tube and "spun down" to produce a "buffy coat" layer of white cells on top of the sedimented red cells. This layer is removed, along with the top approximately one millimeter of red cells, with a sterile Pasteur pipette, added to the culture medium, and dispersed by vigorous aspiration. The culture medium consists of ten

milliliters of medium TC199 (with penicillin and streptomycin added), four milliliters of human or calf serum, and about 0.3 ml of phytohaemagglutinin (Difco, type M). The culture is then incubated at 37°C for approximately three days.

A great many variations on this method are possible. The phytohaemagglutinin may be added directly to the blood sample and gravity or low-speed centrifugation used to sediment the resulting red cell aggregates, leaving the white cells in suspension in the serum, which is then removed and added to the culture medium. Alternatively, the white cells may be separated by means of dextran solutions. Some authors feel that an attempt should be made to include as few red cells in the culture as possible, but in my experience the presence of red cells does no harm, and may actually help later, when the culture is being fixed. Many authors have recommended counting the white cells, and adjusting their final concentration in the culture to some particular value. Many use the serum from the donor's blood sample to make up the medium serum requirement. In general, however, it has been my experience that as long as the required white cells are cultured in a suitable medium in the presence of phytohaemagglutinin satisfactory cultures will result. It should also be noted that various phytohaemagglutinins are sold, and that these may differ in their ability to induce mitosis. It is best to test new lots before placing them in routine use. Also, both the strength of different batches of phytohaemagglutinin and the response of cells from different individuals to the substance seem to vary, so that although it is frequently possible to get by with as little as 0.1 ml of phytohaemagglutinin per culture, it is best to use more, just to be sure.

2. After the required culture time has elapsed, the peripheral leukocyte cultures, just like the bone marrow, should be treated with colchicine (or Colcemid). A few authors prefer to skip the colchicine treatment, but the added number of mitoses collected through its use makes it very desirable except in cases where there is a question of some effect of the drug itself on the parameter being investigated by means of chromosome preparations. Four or five hours treatment

with 10^{-4} *M* colchicine seem to produce acceptable preparations. Generally I add the colchicine from a stock concentrate solution first thing in the morning of the third culture day, and fix the cells first thing in the afternoon. The resulting variation in culture time from one day to the next is usually of no consequence, as there are many mitoses present from about the 48th culture hour on. In more exacting studies, where timing is important, the colchicine is added at 66 hours, and fixation begun at 72 hours.

The cell suspension is removed from the culture bottle and placed in a conical centrifuge tube. This is centrifuged for five minutes in a clinical type centrifuge. The exact time and speed (force) are not important, as long as they are sufficient to sediment the cells. The supernatant medium is removed (a Pasteur pipette is convenient except where the volume is very large) and replaced with ten milliliters of pre-warmed Hanks' or other balanced salt solution (BSS). Care should be taken that the pH of the BSS is near seven. The cells are re-suspended by aspiration with the pipette, and an effort made to break up any cell clumps as much as possible. The resulting suspension is again centrifuged for five minutes. The main purpose of this wash with BSS is to remove most of the serum from the culture, as it would otherwise be fixed along with the cells and result in "dirty" preparations. All but the last milliliter of BSS is removed from over the cell pellet, which is re-suspended again with the pipette. Pre-warmed distilled water is then added slowly with constant agitation. In this way the cells are placed in a hypotonic environment consisting of one-quarter strength BSS, and allowed to swell. The suspension is placed in the incubator at 37°C for ten minutes and then centrifuged for an additional ten minutes. The cells are thus in the hypotonic environment for a total of about twenty minutes. After centrifugation all of the supernatant is carefully removed, without disturbing the pellet of cells. About five milliliters of acetic alcohol (three parts absolute methyl alcohol: one part of glacial acetic acid) is gently added, again without disturbing the cell pellet. The cells are allowed to fix for

about half an hour, after which the pellet is thoroughly broken up by repeated aspiration with a Pasteur pipette. The cells are centrifuged out of the fixative and washed three times by repeated centrifugation and resuspension in fresh fixative. After the final wash, they are re-suspended in a small volume of fresh fixative, generally one-half to one milliliter.

A common variation is the use of 0.7 to 1.0% sodium citrate instead of dilute BSS for the hypotonic treatment. This treatment does seem to help to prevent cell clumping where this is a problem. Various authors recommend the use of ethyl alcohol instead of methyl alcohol for the fixative. Others recommend immediate dispersion of the cell pellet in the fixative. In my experience, while the use of ethyl alcohol makes little difference, the mixing of cells and fixative immediately usually yields preparations of poorer quality than allowing the pellet to fix in place first. Some importance is attached by some to the temperature of the fixative, which it is usually said should be cold. While this in my experience has little effect, it does appear important to be sure that there is not any appreciable amount of water in either the alcohol or the acetic acid; generally it is a good idea to make fresh fixative for each day's use.

3. The fixed cells must next be mounted on microscope slides. The slides should be washed carefully and left in distilled water. As the cell mounting technique is a spreading and flattening one, it is important that the glass surface be really clean and free of grease and oil. It should, in fact, be wettable, just like the surfaces of tissue culture containers, and for much the same reasons. A wet slide is taken from the water, and, while it still bears a film of water, on the surface, a drop of the suspension of cells in fixative is dropped on. The water film will break when the fixative hits it, and the water, and, while it still bears a film of water on the cells stranded on the slide's surface when it gets thin enough. As the film evaporates further, the cells will become remarkably flat, and will adhere firmly to the slide. As the water is pushed away by the fixative film, it will pile up around the edge, where it may be wiped off with a tissue. Care should

be taken, of course, not to disturb the cells in the process. The slides are then allowed to dry completely in air. The process may be hastened by the use of a stream of warm air, as from an ordinary ladies' home hair dryer.

The first slide or two that are made should be checked by making a temporary wet mount with stain or with a phase microscope, if one is available. If it is found that the cells are too closely spaced, the suspension should be diluted with more fixative. Too thick a suspension will prevent the chromosome figures from spreading out as well as they should. If the cells are found to be too sparse, of course, the suspension may be concentrated with the centrifuge.

Fixed cell suspensions may be stored in a refrigerator for later use. If they are to be stored for long, however, it is best to replace the fixative with 70% ethyl alcohol. My own experience has been that stored material never yields slides as good as those prepared immediately, and I always try to prepare an adequate number as soon as possible after the suspension is prepared.

4. An important variation which is particularly useful for infant subjects has been developed by ARAKAKI and SPARKES (1963). Instead of separating the white cells from a large sample of blood in order to prepare the culture, a sterile sample of eight or ten drops of blood is collected from a simple puncture and added directly to the culture medium. From there on the culture is handled in the usual way. The yield of mitoses is, naturally, smaller than from larger cultures, but it is usually more than adequate for diagnostic purposes. The savings in time and medium (about five milliliters sems to be adequate) will probably lead to much wider adoption of this technique in the future.

D. *Tissue cultures.*

Because of the ease with which chromosome preparations can be made by the peripheral blood and the bone marrow techniques, the use of the more classical type of tissue culture has become uncommon in human cytogenetics. There are

occasionally, however, very good reasons for using such cultures. This is the only way, for example, that various tissues or various sites can be sampled in suspected cases of mosaicism. There are a wide variety of different methods for starting tissue cultures. One of the oldest, and one suitable for almost any sort of tissue, is the plasma clot technique. The clot provides an excellent matrix for outgrowth of cells from the original piece of tissue, and cells from the outgrowth may be used to make monolayer cultures in later passages.

1. A sterile skin (or other tissue) biopsy is minced into pieces about 0.5 mm on a side in a small sterile Petri dish containing a litle BSS. A pair of scalpels, used scissors-fashion, is a convenient way to do the mincing. Sterilization of the skin surface for a biopsy should be done, incidentally, with alcohol or a similar organic antiseptic, since iodine or heavy metal antiseptics may be transferred to the culture and poison it. A few drops of chick plasma are streaked on the surface of a cover slip in a Leighton tube or similar container by means of a Pasteur pipette with a bent tip. About four tissue fragments are picked up in *another* pipette in a drop or two of chick embryo extract or thrombin solution and also placed on the surface of the cover slip. The tissue fragments are arranged on the cover slip as quickly as possible with the aid of a rod or pipette tip; this must be done before the plasma begins to gel. The clot is allowed to harden for an hour or two, and then one or two milliliters of TC199, supplemented with penicillin and streptomycin and with 15% foetal or newborn calf serum, is added gently so as to avoid dislodging the clot. The tube is stoppered and placed in the incubator at 37°C. Twice each week the old medium is removed from the culture and fresh is substituted.

2. When a good "halo" of cells has grown out from the explants (generally about the second week) the culture may be "harvested". A cautious procedure is to use some of the cultures to prepare new second-passage cultures, and to fix the rest. Preparation of new cultures can be done either by cutting up the zone of outgrowth and using the pieces to make new plasma clot cultures, or by giving the culture a

fifteen minute's digestion with 0.05% trypsin solution in BSS (preferably a special BSS made up without any calcium or magnesium) in order to produce a cell suspension which is then used to seed new monolayer cultures. The suspension of cells in trypsin may simply be diluted with fresh growth medium and used to plant suitable containers. The cultures to be fixed should, of course, be subjected to a few hours' colchicine treatment before fixation. Adding colchicine to a final concentration in the medium of about 10^{-4} *M* about five hours before the cells are to be fixed gives good results.

3. The colchicine-treated cultures, whether grown on cover slips or, for later passages, as monolayers on the surface of a bottle, must be treated with hypotonic saline before fixation. The medium is poured off and a suitable amount of pre-warmed one-fourth strength BSS substituted. Cover slip cultures are treated for about twenty minutes before fixation. In the case of monolayer cultures, the flask or bottle is shaken violently after about fifteen minutes in order to dislodge the dividing cells. The resulting cell suspension is then placed in a centrifuge tube and handled exactly like leukocyte cultures, as described above. Cover slip cultures are first "pre-fixed" for a few seconds in a mixture of a few drops of fixative or acetic acid in hypotonic BSS, and then immersed in full strngeth acetic alcohol fixative. The brief treatment with very dilute acid seems to help to swell the cells, thus producing better spread chromosome preparations. Care should be exercised, however, not to keep the cells in this solution too long, as the cells will be broken and the chromosomes either lost or spread over the entire coverslip. Care should also be used in handling the cover slip during these steps, as the dividing cells are much less firmly attached than interphase cells, and can quite easily be torn loose by rough handling. The cover slips are allowed to fix completely in three five or ten minute changes of fresh fixative and then laid out, cell side up, to air dry. The original explants, if they are still present, should be carefully removed at this time, as their thickness will otherwise interfere with mounting.

EXAMINATION

A. *Staining and mounting.*

A wide variety of stains have been used for human chromosomes. The most widely used is undoubtedly aceto-orcein, long a favorite of the insect cytogeneticist. The Feulgen reaction, although more complicated, has also been used often, and produces very good material. Perhaps because they were already familiar with them, many authors have used ordinary haematological stains, such as Geimsa. Except for the Feulgen reaction, all may be used without any prior treatment of the preparations. In a number of laboratories, however, a mild acid hydrolysis is given before staining with aceto-orcein or Geimsa, in order to prevent light staining of cytoplasm and debris, which some find objectionable. Although I prefer Feulgen preparations, the ease and speed with which aceto-orcein staining can be accomplished has led me to use it almost exclusively.

Orcein from various manufacturers, and even from different lots from the same manufacturer, may vary a great deal. The synthetic orceins generally seem to be the best. The staining mixture is prepared by adding two grams of synthetic orcein (Gurr) to 50 ml of hot glacial acetic acid (be careful that it does not boil over when the orcein is added, staining everything in the vicinity). When this mixture has cooled to room temperature it is diluted with 50 ml of distilled water, producing a 2% solution of orcein in 50% acetic acid. The orcein granules will not be completely dissolved, and must be filtered out by passing the mixture through a hard filter paper. As the stain will also crystallize out during storage, it is best to refilter the solution from time to time. Otherwise some particles will deposit on the surface of the slides during staining, resulting in "dirty" looking preparations. For temporary slides, as for example when staining to check on cell concentration in the fixed cell suspension, a drop of stain is simply placed on the slide and covered with a cover glass. If such slides are to last more than a few minutes, however,

the edges of the cover glass must be sealed with hard wax or cement. Permanent slides are stained by immersing them in the stain in a Coplin jar or similar staining dish for about fifteen minutes. Cover slip preparations are easily stained using a Chen staining rack.

The stained slides are rinsed briefly in three changes of absolute ethyl alcohol. It is important that the alcohol be kept from becoming acid, as it will then destain the chromosomes to an objectionable degree. A drop or two of dilute sodium hydroxide solution added to the alcohol will prevent this. The rinsed slides (or cover slips) may either be mounted directly with an alcohol-miscible medium such as Euparal, or allowed to air dry first and then mounted in other media such as Canada balsam or Permount.

B. *Autoradiography.*

Autoradiography of cells labeled with tritiated thymidine is becoming more common in human cytogenetics, and is sometimes useful to help identify particular chromosomes, particularly the X chromosome (MORISHIMA, et al., 1962). The extremely short range of the beta particles emitted by tritium results in the production of silver grains in the developed emulsion very close to the location of the radioisotope in the cell. The tritium-labeled deoxyribose nucleic acid (DNA) precurser is usually supplied to the cells at the very end of the DNA synthetic period. With this so-called "late labeling" technique only chromosomes or chromosome regions which finish their DNA synthesis very late incorporate the labeled thymidine, and consequently produce silver grains in the emulsion above. For leukocyte cultures, the labeled compound may be added about five hours before the cells are to be fixed.

1. Tritiated thymidine is added to the culture medium to a final concentration of about 0.5 μC/ml (the exact amount will depend both on the specific activity of the labeled compound and the time one is willing to wait for adequate exposure

of the emulsion) (*). The culture is then colchicine-treated and handled just like any other leukocyte culture, except for the disposal of the now radioactive medium.

2. After the cells have been mounted on slides in the usual way, they must be covered with a photographic emulsion. I find liquid "dipping" emulsions much easier to handle than stripping film. Nuclear Track Emulsion NTB (Kodak) is opened and melted in the darkroom (it is only too easy to forget that one is dealing with a photosensitive emulsion which cannot be exposed to light!) in a water bath at about 40°C. While the emulsion is becoming completely liquified, the slides are soaked in warm distilled water to hydrate the ecIls. The slides are then dipped briefly into the emulsion and stood on end to drain and dry. Once dry, they are stored in a light-tight container in a refrigerator. It is important to keep the emulsion cold as much as possible before it is developed, because storage at higher temperatures will increase the number of background silver grains.

3. After the emulsion has been adequately exposed, which generally takes about one week, the slides are developed in the same manner as photographic plates. A series of staining dishes with a removable slide carrier of glass is convenient for this procedure. The slides are developed for two minutes in D-11 developer (Kodak), rinsed in water, and fixed in acid fixer (Kodak) for five minutes. They are then washed in running water for fifteen or twenty minutes, and allowed to dry. It is a good plan to develop one or two of a batch of slides first, as a test; the rest may then be left to expose longer if there are too few grains on the test slides.

4. Most of the common chromosome stains are unsuitable for use on autoradiographic preparations, as they stain the emulsion itself, as well as the chromosomes. The chromosomes

(*) Although the radioisotope tritium emits only a very low energy beta particle, and thus does not present any great hazard outside of the body, it is certainly very dangerous if ingested, because of its incorporation into a DNA precurser. It should never be pipetted by mouth. Local regulations on the handling and disposal of radioisotopes must, of course, be observed.

may be stained through the emulsion with only a moderate staining of the emulsion, however, with Geimsa diluted 3:1 with phosphate buffer at pH 7. Generally the stain is diluted directly on the slide, as is commonly done with blood smears. The staining mixture is left on the slide for two or three minutes, and then rinsed off with water. After the slide has been air dried, a cover glass may be mounted in the usual manner. The emulsion on the back surface of the slide may be scraped off with a razor blade if it is found objectionable.

C. *Microscopy.*

Most human cytogeneticists use the ordinary bright-field compound binocular microscope for chromosome analysis. Since the chromosomes are quite small, it is naturally desirable that the microscope be capable of a high degree of resolution. A low power objective lens of about 10 X magnification, with a working distance of several millimeters is desirable. Slides may then be scanned for figures suitable for analysis without the bother of removing the immersion oil from the surface every time the high power objective is used. A high power oil immersion objective of good quality with a magnification of about 100 X is used for detailed examination of figures identified y low power scanning. These two objectives are sufficient for most work. Oculars of about 10 X are suitable, giving about 1,000 X total magnification with the oil immersion objective. The oculars should naturally be corrected for the objective lenses used. A graduated mechanical stage should be provided because it is frequently desirable to return to a figure already examined, and the stage graduations provide the only really satisfactory means of doing so. The substage condenser should be fully corrected, and have as high a numerical aperature as the high power objective used, in order that the full resolving power of the objective may be used when required. Although the oiling of the condenser front lens required to realize numerical aperatures greater than one is generally considered too much trouble for routine examination, the extra resolving power will occasionally be found

invaluable. It is worth while to devote considerable attention to the microscope's illuminating system. Even the very best optics will produce very inferior images if the whole optical system, including the lamp and the field condenser, is not properly aligned and focused. With many chromosome stains, including particularly orcein, it is desirable to use a green filter to reduce eye strain and increase contrast. A medium green, such as a Wratten number 58 (Kodak) is suitable.

A number of cytogeneticists prefer to use phase contrast for chromosome examination. A medium-bright contrast is suitable. Such objectives and condensers are available for most microscopes. One advantaeg of phase contrast is that staining is not critical, and may in fact be eliminated entirely. On the other hand, some resolution is lost with a phase system. Also, it is sometimes impossible to tell whether an object in the field is chromosomal or not, using phase contrast; color contrast in bright field will frequently answer the question.

D. *Photography.*

At present, human cytogeneticists depend heavily on photographic techniques. It is unfortunate that the photomicrographs are frequently of poor quality. Photographs are always used for the preparation of karyotypes, an important element in much human cytogenetic work. Although I personally feel that it is a poor practice, in some laboratories photographs are actually used for all chromosome analyses, and the microscope is used only to find suitable cells and photograph them.

Most microscope manufacturers provide equipment to outfit their instruments for photomicrography. These vary from simple bellows and film-holder outfits to elaborate automatic equipment. Fortunately, since chromosome photographs are usually made under a very standardized set of conditions of illumination, color, and magnification, even very simple equipment is adequate. Most workers use 35 mm roll film, and with modern fine grain emulsions and developers this size is generally quite suitable. Provided of course that

the microscope itself is producing an optimum image, focus, vibration, and contrast are the important considerations. Fuzzy pictures may be caused either by poor focus (the fact that the image is focused on a viewing screen does not always mean it is focused on the film plane), or by vibration. It is worth noting that shutter mechanisms may introduce enough vibration to cause trouble. Sufficiently high contrast should be achieved through use of a high contrast emulsion, such as Kodak High Contrast Copy Film, and, if necessary, high contrast development. Daylight-loading 35 mm processing tanks are a great convenience, and enable one to entirely dispense with a darkroom for the processing of films.

Enlargement is, of course, necessary for printing 35 mm negatives. Some sort of darkroom is necessary for exposing, developing, and fixing the enlargements. Small laboratories may find it advantageous to have print work done by a general photographic workshop. In this case, it is particularly important that the contrast of the negatives supplied be sufficient to allow ordinary printing on medium contrast paper such as Kodabromide F2 or F3 (Kodak). Although one may be fortunate enough to have one's work done by someone interested and capable enough to use special techniques, it is best not to count on it. Of course, if printing is done by the cytogenetics laboratory, special efforts may be made to "rescue" poor negatives. Dodging, "burning in", and other special techniques may sometimes be helpful. Very high contrast papers and development are useful also. Care must be exercised, however, to avoid losing chromosome areas or changing their dimensions through "blooming". As a general rule, it is much better to work toward the production of good, sharp, high contrast negatives in the first place than to try to salvage those of poor quality in the printing.

E. *Karyotype preparation.*

A karyotype is generally made from cut out chromosomes of an enlargement showing the chromosomes at a total magnification of 2,000 to 4,000 diameters. The cut-outs are sorted,

paired, and attached to a sheet of paper or cardboard. Fig. 2 is such a "paste-up" prepared from the photograph shown in Fig. 1. There is no standard for the arrangement of karyotypes on the sheet; various laboratories have adopted systems of their own. For example, while I find it easier and more pleasing to place the chromosomes with their short arms down, most workers prefer to put them the opposite way. It is convenient, whatever arrangement is adopted, to have some standard pattern, which may take the form a stencil used to mark karyotype sheets with the chromosome pair locations, or a printed form, such as was used for Fig. 2.

The chromosomes are cut from the print with small scissors, taking care not to amputate parts of any of the chromosomes. It some of the chromosomes in a figure overlap, as they frequently do, two prints of the same figure may be used, cutting one of the overlapping chromosomes from each. The cut-outs may be sorted and test matched with fine-pointed forceps. When they are all paired to the investigator's satisfaction, they are attached permanently to the kayotype sheet with glue, paste, or tape. I personally prefer to coat both the sheet and the backs of the cut-outs with rubber cement, and to stick the chromosomes down after the cement has dried.

It is convenient to make all karyotypes in a form suitable for use in a publication, rather than having to re-do them specially for the purpose if it is decided later that they are to be used. Half-tone cuts are extremely expensive to make, and the cost is proportional to their area. A very careful use of space is thus manditory. While the chromosomes need not be put so close together that it is difficult to distringush pairs, many karyotypes are prepared with the chromosomes separated by excessive empty spaces. Placing the sex chromosomes or an aberrant chromosome way out on the edge of the sheet may actually double the cut area, and thus the cost. An effort should be made to use prints with practically white backgrounds, and to use a white paper or board of good quality and surface texture for the backing. It may thus be possible

for the publisher to entirely eliminate the annoying cut-out edge image from the the reproduction.

NORMALITY

A. *The "normal" human karyotype.*

It is not, perhaps, entirely out of place in a paper dealing with the methods of human cytogenetics to discuss the concepts of normality and abnormality. Several times groups have met and attempted to define exactly what constitutes a normal human karyotype. The criteria that have envolved are, not

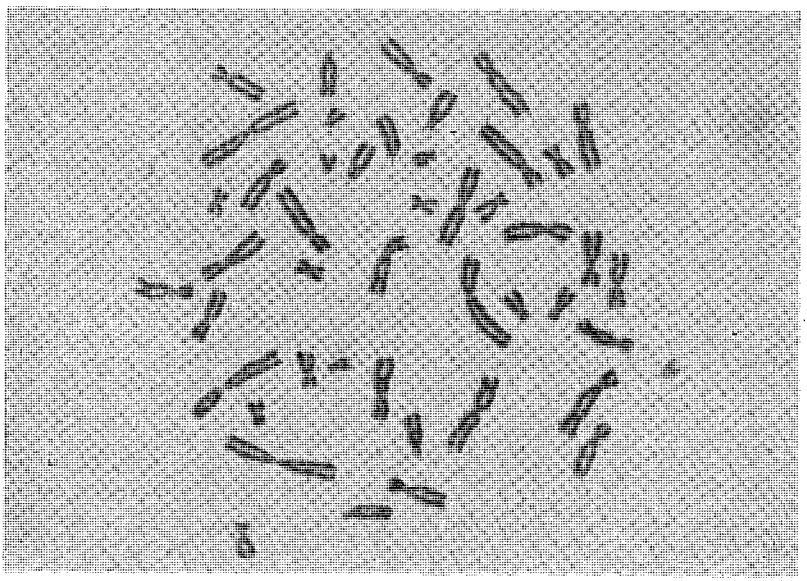

Fig. 1. Chromosome spread from a cultured leukocyte from a normal man.

unexpectedly, statistical, rather than absolute. The most recent meeting was known as the London Conference (1963). The task is a difficult one. Only a few pairs of chromosomes were generally agreed to be identifiable in an absolute sense on

the basis of relative length and centromere position. Refering to Fig. 2, these are pairs 1, 2, and 3. It is generally felt that the rest are identifiable only by groups in any single figure. It is possible to identify four chromosomes as belonging to the 4-5 (B) group with a high degree of certainty, for example, but which are the number 4 chromosomes and which are the number 5's cannot be said with certainty. This is, of course,

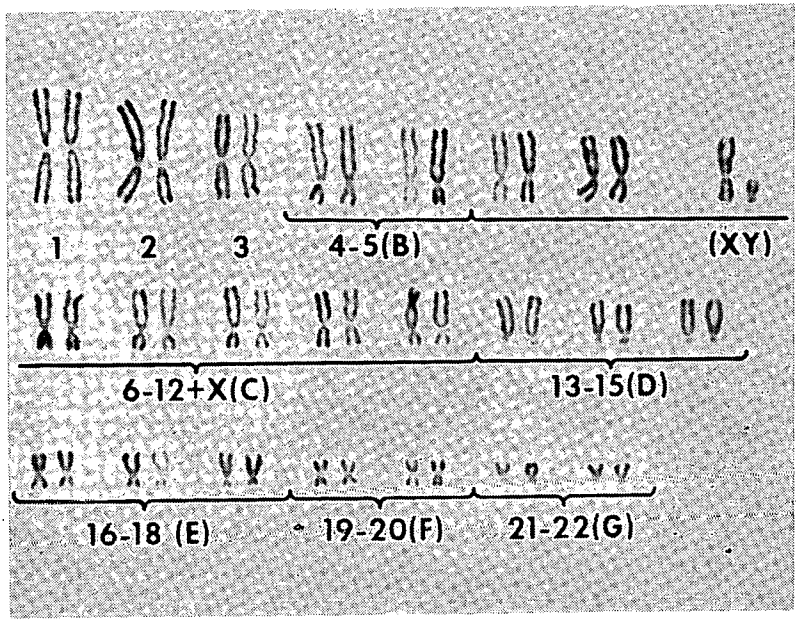

Fig. 2. Karyotype constructed from the photograph shown in Fig. 1.

because the variability in length and centromere position, though small, is large in comparison to the differences between the pairs. In other words, the pairing of chromosomes within groups in a karyotype *does not necessarily coincide with genetic homolgy.*

It had been hoped that autoradiography, which has revealed differences between chromosomes in the time at which DNA synthesis is completed, would provide an answer to this dilema.

Unfortunately, it has notyet done so. It is only possible to be sure of the constancy of DNA replication patterns for chromosomes which can be identified by means of some independent criterion. Specific chromosomal abnormalities will probably eventually make such identification possible for the whole human chromosome complement. To date, however, only a few useful abnormalities have been found. It is interesting, in this connection, to reflect that in some cases chromosome identification depends on an abnormality in a circular fashion. Thus of the four small acrocentric chromosomes in the 21-22 (G) group (five, including the Y in the male), chromosome 21 is, by definition, that chromosome which is present in triplicate in mongolian idiots. Which one is extra, and which are its homologues in a given cell, is impossible to say.

B. *Variations in number and form.*

As mentioned at the beginning, variations in the number and form of the chromosomes of individuals or of individual cells are the subject of investigation in human cytogenetics. It is essential, however, to recognize that spontaneous variation occurs, and is, in fact, common. The presence of an abnormality in a figure does not *necessarily* have any relevence to the agent or condition being studied. The *normal* number of chromosomes in human somatic cells is 46. In peripheral leukocyte cultures, however, one always finds some figures which do *not* have 46 chromosomes. The frequency of such figures varies from culture to culture, and from laboratory to laboratory, but may run as high as 10 or 15% in normal cultures from normal individuals. It is thought that these aneuploid figures are largely the result of mechanical cell breakage and chromosome loss during preparation. This idea is supported by the fact that most of the aneuploid figures have less than 46 chromosomes, and only a few have more than 46. Further, karyotype analysis usually shows that which one is lost is more or less random. Therefore, if it is thought that a patient's condition is in some way associated with chromosomal mosaicism, with

some cells normal and some missing a particular chromosome, it is not enough to have demonstrated that some of the cells have 45 chromosomes. It is also necessary to show that it is consistently one particular chromosome that is missing.

There are certain features of the human chromosomes which can be confused with abnormalities. For example, the chromosome usually called number 9 is known to have a secondary constriction which is occasionally striking enough to be mistaken for a break. Similarly, there is a tendency for the acrocentric chromosomes of the 13-15 (D) and 21-22 (G) groups to associate by their short arms. The inexperienced observer can easily mistake two chromosomes associated in this way for a single chromosome. Further, the acrocentric chromosomes sometimes show very definite satellites on their short arms, which could, of course, be mistaken for small fragments.

Another form of normal chromosome variation is the phenomenon of spontaneous chromosomal aberration. Chromosomal breakage, and more rarely recombination, occur in the absence of any obvious cause. Here again there is a great deal of variation between cultures and between laboratories. In normal peripheral leukocyte cultures, for example, simple breaks of one or both chromatids of a chromosome are seen in several percent of the figures. Without some experience, these acentric fragments may not be recognized for what they are, and may be acored as extra chromosomes, leading to erroneous counts.

CONCLUSION

I have attempted to give, in very brief form, an outline of some of the techniques now in use in the field of human cytogenetics, as well as some comments on some of the more frequently encountered problems. It is obviously very far from being a complete guide. Nevertheless, I hope that some will find it helpful. In addition, it may perhaps help others to better understanding of this new but already very significant

field, which has been so largely shaped by its peculiar technical problems, and by the solutions which have been found for them.

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REVISÃO DE LIVROS

FERMENTDIAGNOSTIK INTERNER ERKRANKUNGEN (Diagnóstico Enzimático das Doenças Internas) — Diethard Amelung — Georg Thieme Verlag, Stuttgart, 1964.

Da autoria do Dr. Diethard Amelung, Docente da I Clínica Médica da Academia de Medicina de Dusseldorf, êste compêndio de 173 páginas, com 37 figuras ilustrativas do texto, estuda em profundidade a natureza dos fermentos orgânicos e a patogenia das suas variações no sôro. Justificando o título do livro, estuda o autor a aplicação diagnóstica das referidas variações em muitos setores da patologia, tais como: doenças cárdio-vasculares, hepatopatias (cirrose e hepatites), doenças das vias biliares, icterícias (diagnóstico diferencial), pancreatites agudas, nefropatias, doenças infecciosas, hemopatias, neoplasias e doenças musculares.

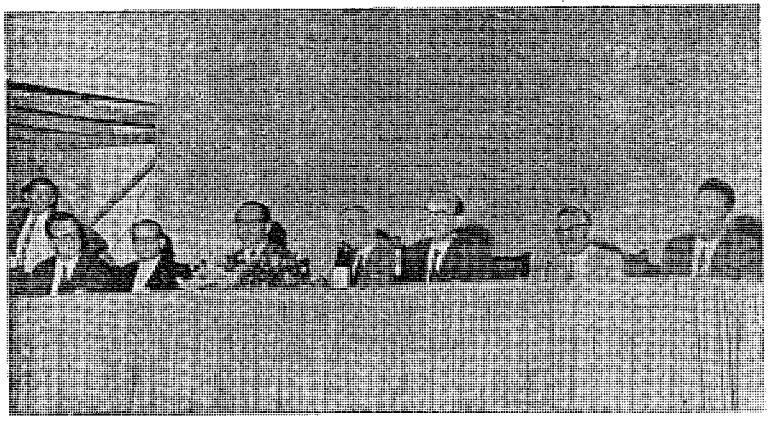
Trata-se de uma obra escrita em linguagem concisa, valiosa para os que estudam os problemas da físio-patologia humana e útil para os clínicos que nela encontrarão compendiadas tôdas as aquisições dêsse moderno campo de diagnóstico laboratorial.

NOTICIÁRIO

VI CONGRESSO BRASILEIRO DE ENDOCRINOLOGIA E METABOLOGIA

Com a participação de 276 congressistas de todo o Brasil, realizou-se na Faculdade de Medicina da Universidade de São Paulo o VI Congresso Brasileiro de Endocrinologia e Metabologia patrocinado pela Sociedade Brasileira de Endocrinologia e Metabologia.

A Comissão Organizadora do Congresso foi presidida pelo Professor Emilio Mattar e constituída pelos Drs. Licio Marques



Mesa que presidiu a solenidade de abertura do VI Congresso Brasileiro de Endocrinologia e Metabologia. Sentados da esquerda para a direita: Drs. Luiz Carlos Lobo, Ahron Hutz, Emilio Mattar, João Alves Meira, A.B. Ulhôa Cintra e Marcionilio Lins

de Assis, Walter Bloise, Arnaldo Sandoval e Julio Timoner, tendo sido o congresso estruturado de modo a permitir divulgação dos progressos mais recentes da endocrinologia e discussão dos assuntos mais controversos da especialidade.

A solenidade inaugural foi presidida pelo Professor João Alves Meira, representando o Magnífico Reitor da Universidade de São Paulo. Após a saudação de abertura do Professor Emilio Mattar, presidente da S.B.E.M., o Professor A.B. Ulhoa Cintra proferiu conferência sôbre tema da maior atualidade: Reforma Universitária.

O conclave congregou a maior parte dos especialistas nacionais e contou com a participação de endocrinologistas da Colômbia, Equador e Paraguai. Em 5 dias de atividade discutiram-se quase tôdas as questões de relêvo da atualidade endocrinológica, tendo sido apresentadas grande parte das pesquisas da especialidade em andamento no Brasil.

VI CONGRESSO PAN-AMERICANO DE ENDOCRINOLOGIA

O VI Congresso Pan-Americano de Endocrinologia realizar-se-á na cidade do México, na Unidade de Congressos Médicos do Centro Médico do Instituto Mexicano de Seguro Social, de 10 a 15 de outubro de 1965.

As línguas oficiais do congresso serão o espanhol e o inglês; haverá facilidades para tradução simultânea em 3 das 6 salas de reuniões que serão utilizadas. As comunicações feitas em português ou em francês serão traduzidas para as duas línguas oficiais.

Resumos de trabalhos a serem submetidos à apreciação da Comissão Organizadora (máximo de 200 palavras) para apresentação no Congresso deverão ser enviados até 1.º de maio de 1965 à Secretaria Geral. A seleção final dos trabalhos será feita pelos presidentes das diferentes sessões até 15 de maio de 1965.

Um cuidadoso programa social está sendo organizado para todos os participantes. A taxa de inscrição para membros titulares do Congresso será de US$ 30.00; será cobrada taxa de US$ 20.00 para acompanhantes.

Fórmulas para registro, remessa de trabalhos e outras informações poderão ser obtidas com o Secretário Geral do Congresso:

Dr. Carlos Gual
Instituto Nacional de la Nutrición
Calle del Dr. Jimenes 261
México 7, D.F., México

Reservas de hotéis, informações sôbre excursões e viagens poderão solicitadas diretamente a:

P.A.C.E. Official Congress Headquarters
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U.S.A.

Facilidades serão concedidas aos participantes de um vôo especial Buenos Aires-México-Buenos Aires (via Santiago do Chile).

O programa preliminar do Congresso é o seguinte:

I — Dezesseis conferências especiais de 30 minutos de duração:

— CONFERÊNCIA INAUGURAL
Dr. Bernardo A. Houssay (Argentina)

— CONTRÔLE DA BIOSÍNTESE DOS HORMÔNIOS ESTERÓIDES
Dr. Ralph I. Dorfman (E.U.A.)

— DIABETES MELLITUS: ASPECTOS SOCIAIS E BIOLÓGICOS
Dr. Salvador Zubirán (México)

— PROBLEMAS ENDÓCRINOS NA GERONTOLOGIA DA REPRODUÇÃO
Dr. Alejandro Lipschutz (Chile)

— HORMÔNIOS KININAS
Dr. Mauricio Rocha e Silva (Brasil)

— METABOLISMO DO GLICOGÊNIO E SUA REGULAÇÃO
Dr. Luis F. Leloir (Argentina)

— CONTRÔLE HORMONAL DA REPRODUÇÃO
Dr. Gregory Pincus (E.U.A.)

— HIPERFUNÇÃO ADRENO-CORTICAL
Dr. George W. Thorn (E.U.A.)

— HORMÔNIO DE CRESCIMENTO E PEPTIDES SEMELHANTES DE ORIGEM PITUITÁRIA
Dr. John C. Beck (Canadá)

— CONCENTRAÇÃO DE OCITOCINA NO SANGUE JUGULAR DURANTE O PARTO E A LACTAÇÃO
Dr. Roberto Galdeyro-Barcia (Uruguai)

— MECANISMOS PARA O CONTRÔLE E SECREÇÃO DA ALDOSTERONA
Dr. Frederic C. Bartter (E.U.A.)
— FUNÇÃO DO CROMOSOMA SEXUAL E SUAS ANORMALIDADES
Dr. Melvin M. Grumbach (E.U.A.)
— LUZ, CÉREBRO E METAMORFOSE
Dr. Carrol Williams (E.U.A.)
— REGULADORES QUÍMICOS DA DIVISÃO CELULAR
Dr. Albert Szent Gyorgi (E.U.A.)
— EFEITO DOS ESTROGÊNIOS SÔBRE A EXPRESSÃO GENÉTICA
Dr. Roy O. Greep (E.U.A.)

II — Oito simpósios constituídos cada um de cinco trabalhos de 20 minutos, seguidos de uma discussão geral de 40 minutos de duração:

— INDUÇÃO E INIBIÇÃO DA OVULAÇÃO
Coordenadores: Dr. Celso R. Garcia (E.U.A.)
Dr. Jorge Martínez-Manautou (México)
— PARATIREÓIDES E OSSOS
Coordenadores: Dr. A.B. Ulhoa Cintra (Brasil)
Dr. Howard Rasmussen (E.U.A.)
— FISIOPATOLOGIA DOS QUADROS CLÍNICOS DA VIRILIZAÇÃO
Coordenadores: Dr. Carlos, Gual (México)
Dr. Mortimer B. Lipsett (E.U.A.)
— ASPECTOS HORMONAIS DO EDEMA E DA HIPERTENSÃO
Coordenadores: Dr. John H. Laragh (E.U.A.)
Dr. Frederico Dies (México)
— PREDIABETES
Coordenadores: Dr. Jerome W. Conn (E.U.A.)
Dr. Virgilio G. Foglia (Argentina)
— HORMÔNIOS PROTEICOS
Coordenadores: Dr. Choh Hao Li (E.U.A.)
Dr. Klaus Hofman (E.U.A.)
— PROGRESSOS NO ESTUDO DA FUNÇÃO TIREODIANA
Coordenadores: Dr. Arturo Atria (Chile)
Dr. Edwin B. Astwood (E.U.A.)
— TESTÍCULO HUMANO NORMAL E PATOLÓGICO
Coordenadores: Dr. Felipe A. de la Balze (Argentina)
Dr. Roberto A. Mancini (Argentina)

III — Trinta e seis sessões de comunicações curtas sôbre temas selecionados, que consistirão de 5 a 10 trabalhos de 10 minutos, seguidos de 5 minutos de discussão:

— PREDIABETES
Coordenadores: Dr. Rafael A. Camerini-Dávalos (E.U.A.)
Dr. Oscar Lozano-Castañeda (México)
— INCIDÊNCIA DE DIABETES NO CONTINENTE AMERICANO
Coordenadores: Dr. Juan A. Rull (México)
Dr. Leo P. Krall (E.U.A.)
— MECANISMO DE AÇÃO DE DROGAS HIPOGLICEMIANTES
Coordenadores: Dr. Alexander Marble (E.U.A.)
Dr. Emilio Mattar (Brasil)
— MECANISMOS DE REGULAÇÃO DA INSULINA
Coordenadores: Dr. Harry N. Antoniades (E.U.A.)
Dr. Rafael Rodrigues (México)
— BÓCIO ENDÊMICO NAS AMÉRICAS
Coordenadores: Dr. Jorge Maisterrena (México)
Dr. John B. Stanbury (E.U.A.)
— BIOSÍNTESE DOS HORMÔNIOS TIREOIDIANOS
Coordenadores: Dr. Jacob Robbins (E.U.A.)
Dr. Roberto Soto (Argentina)
— TRATAMENTO DO HIPERTIREOIDISMO
Coordenadores: Dr. Pablo Fletcher (Panamá)
Dr. Francisco de Venanzi (Venezuela)
— CÂNCER DA TIREÓIDE
Coordenadores: Dr. Luiz Carlos G. Lobo (Brasil)
Dr. Sidney C. Werner (E.U.A.)
— MECANISMOS DE AÇÃO DOS HORMÔNIOS
Coordenadores: Dr. Fernand Péron (E.U.A.)
Dr. Bernardo L. Wajchemberg (Brasil)
— ENDOCRINOLOGIA COMPARADA
Coordenadores: Dr. Howard A. Bern (E.U.A.)
Dr. Paulo Sawaia (Brasil)
— ASSOCIAÇÃO E TRANSPORTE DE HORMÔNIOS
Coordenadores: Dr. Eduardo Gaitán (Colômbia)
Dr. Richard Doe (E.U.A.)
— ENDOCRINOLOGIA FETAL
Coordenadores: Dr. Leonard Axelrod (E.U.A.)
Dr. Kurt Benirschke (E.U.A.)
— ASPECTOS ENDÓCRINOS NA ALTITUDE
Coordenadores: Dr. Rodrigo Fierro (Equador)
Dr. Federico Moncloa (Perú)
— RITMOS HORMONAIS
Coordenadores: Dr. Franz Halberg (E.U.A.)
Dr. Francisco Parra-Gil (Equador)
— ALTERAÇÕES ENDOCRINOLÓGICAS NA DESNUTRIÇÃO
Coordenadores: Dr. Silvestre Frenk (México)
Dr. Alberto Viau (Guatemala)

— PEPTIDES COM ATIVIDADE HORMONAL
Coordenadores: Dr. Hector Croxato (Chile)
Dr. Aaaron Lerner (E.U.A.)
— GONADOTROPINAS
Coordenadores: Dr. Alexander Albert (E.U.A.)
Dr. Eugenia Rosemberg (E.U.A.)
— METABOLISMO DOS HORMÔNIOS ESTERÓIDES EM TECIDOS ENDÓCRINOS
Coordenadores: Dr. Lewis L. Engel (E.U.A.)
Dr. Kenneth D. Savard (E.U.A.)
— METABOLISMO DOS HORMÔNIOS ESTERÓIDES NOS TECIDOS PERIFÉRICOS
Coordenadores: Dr. Rober Guerra-Garcia (Peru)
Dr. Frank Ungar (E.U.A.)
— RELAÇÃO ENTRE A ESTRUTURA MOLECULAR E A AÇÃO BIOLÓGICA DOS HORMÔNIOS
Coordenadores: Dr. Rulon W. Rawson (E.U.A.)
Dr. Howard J. Ringold (E.U.A.)
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Coordenadores: Dr. Claude Fortier (Canadá)
Dr. Roger Guillemin (E.U.A.)
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— HORMÔNIOS E METABOLISMO LIPÍDICO
Coordenadores: Dr. Donald S. Fredrickson (E.U.A.)
Dr. Abraham White (E.U.A.)
— MECANISMOS DE AÇÃO E METABOLISMO DA ALDOSTERONA
Coordenadores: Dr. Bernardo Nusimovich (Argentina)
Dr. James A. Tait (E.U.A.)
— ASPECTOS ENDÓCRINOS DA OBESIDADE
Coordenadores: Dr. Jean Mayer (E.U.A.)
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Dr. Francisco Gómez-Mont (México)

IV — Dêz sessões de comunicações curtas sôbre temas diversos, que consistirão de 5 trabalhos de 10 minutos cada um, seguidos de 5 minutos de discussão:

— SESSÃO 1
Coordenadores: Dr. Oscar V. Dominguez (México)
Dr. Leo T. Samuels (E.U.A.)
— SESSÃO 2
Coordenadores: Dr. Kenneth J. Ryan (E.U.A.)
Dr. Ramiro Delgado (Colômbia)
— SESSÃO 3
Coordenadores: Dr. Oscar Hechter (E.U.A.)
Dr. Hernán Acevedo (E.U.A.)
— SESSÃO 4
Coordenadores: Dr. Juan Carlos Penhos (Argentina)
Dr. Domingo Ramirez (Chile)
— SESSÃO 5
Coordenadores: Dr. Procopio Valle (Brasil)
Dr. Ricardo Moreno Azorero (Paraguai)
— SESSÃO 6
Coordenadores: Dr. Roberto Gómez (Chile)
Dr. Samuel Solomon (E.U.A.)
— SESSÃO V
Coordenadores: Dr. Juan J. Staffieri (Argentina)
Dr. M.X. Zarrow (E.U.A.)

— SESSÃO 8

Coordenadores:	Dr. José Cerviño	(Uruguai)
	Dr. Olaf H. Pearson	(E.U.A.)

— SESSÃO 9

Coordenadores:	Dr. Alfredo Jadresic	(Chile)
	Dr. José Ribeiro do Valle	(Brasil)

SESSÃO 10

Coordenadores:	Dr. Luis A. Eguiguern	(Equador)
	Dr. Efraim Otero Ruiz	(Colômbia)

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CORTICOSTERÓIDES

Schering

1 — de ação glicocorticóide

CELESTONE
Comprimidos de 0,5 mg — Frasco com 15
CELESTONE injetável
Ampola de 5,3 mg = 4 mg betametasona álcool
CELESTONE elixir
Frasco com 60 cm^3
CELESTONE colírio
Frasco com 5 cm^3 contendo 1 mg por cm^3
CELESTONE ungüento oftálmico
Bisnaga com 3 g
DERONIL
Comprimidos de 0,5 mg — Frasco com 20
Comprimidos de 0,75 mg — Frasco com 12
DERONIL concentrado
Comprimidos de 2 e 3 mg — Frascos com 10
METICORTEN
Comprimidos de 5 mg — Frasco com 20
METICORTEN concentrado
Comprimidos de 20 e 50 mg — Frasco com 10
METICORTELONA
Comprimidos de 5 mg — Frasco com 20
METICORTELONA concentrada
Comprimidos de 20 e 50 mg — Frascos com 10
METICORTELONA intra-articular
Frasco-ampola de 5 cm^3 contendo 25 mg por cm^3
METICORTELONA intramuscular
Frasco-ampola de 5 cm^3 contendo 20 mg por cm^3
METICORTELONA pomada
Bisnaga com 5 g
METICORTELONA pomada com neomicina
Bisnaga com 5 g

2 — de ação mineralocorticóide

CORTEXON bucaletas
Bucaletas de 2,5 mg — Frasco com 20
CORTEXON injetável
Ampolas de 5, 10 e 25 mg — Estojo com 1

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INSTITUTO DE ENDOCRINOLOGIA

SANTA CASA DA MISERICÓRDIA

CENTRO DE PESQUISAS ENDOCRINOLÓGICAS

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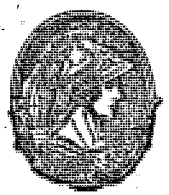

Composto e impresso
na Oficina Gráfica da
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