BRITISH MEDICAL JOURNAL

LONDON SATURDAY SEPTEMBER 1 1951

ACTION OF SEX HORMONES ON EXPERIMENTAL DIABETES*

BY

BERNARDO A. HOUSSAY, M.D.

Director, Institute of Biology and Experimental Medicine, Buenos Aires ;
Foreign Member of the Royal Society

[WITH SPECIAL PLATE]

Total pancreatectomy demonstrated the part played by the pancreas in the pathogenesis of diabetes ; it also made possible the discovery of insulin and of the importance of the hypophysis and the adrenals in the course of the disease. Furthermore, basic knowledge of carbohydrate, protein, and fat metabolism in diabetes has been obtained by the use of this method. These experiments, however, were nearly always performed in dogs and cats—animals in which total pancreatectomy provokes diabetes differing in its intensity, course, and metabolic disturbances from that seen in other species, such as birds, herbivora, and man. Moreover, the influence of many modifying factors cannot be adequately studied in diabetes of so acute and severe a course as that provoked by total pancreatectomy in cats and dogs. It is therefore a sound experimental rule that in order to obtain a good knowledge of this disease it should be studied in several species and under different conditions. Certain types of experimental diabetes with a slower development and longer course lend themselves more readily to the study of the prevention and cure, either early or late, of certain forms of diabetes. A most useful example is the diabetes obtained in rats after subtotal pancreatectomy in which about 95% of the gland has been removed (Foglia, 1944). The course of this type of diabetes in the rat has been ably described by Foglia (1944), and can be divided into three stages:

In the first stage, that of *pre-diabetes* or *unapparent diabetes*, there is no hyperglycaemia or glycosuria, the body weight increases, and there are no symptoms (Table I). It usually lasts one to two months.

TABLE I

	Glycaemia after 7 Hours* Fasting	Glycosuria	Glucose- tolerance Curve	Growth
Pre-diabetes Incipient diabetes	Normal Normal or less than 150 mg./ 100 ml.	No Only post- prandial	Normal High pro- longed	Normal Diminishes or ceases
Manifest diabetes	More than 150 mg./100 ml.	Present	Diabetic	Non- decrease

In the second stage, that of *incipient diabetes*, there is no hyperglycaemia after seven hours of fasting, but there is postprandial glycosuria. Furthermore, the tolerance curve for glucose becomes progressively higher and more prolonged (Foglia, Orlas, and Sara, 1944 ; Foglia, 1945). There

is an increase in the intake of food with a parallel increase in spontaneous activity (Souto-Maior and Foglia, 1944). This stage lasts into the third or fourth month.

In the third stage, that of *manifest diabetes*, there is hyperglycaemia (after seven hours of fasting, more than 150 mg. per 100 ml.) and glycosuria, ketonuria, and polyuria, with loss of body weight, fatty liver, etc. Finally, there is cachexia and death. If the diabetes in an intense form lasts several months, cataracts occur (Foglia and Cramer, 1944); in the kidney there are sclerosis or hyalinosis of the glomeruli (Foglia, Mancini, and Cardeza, 1950), progressive decrease in the phosphatase of the convoluted tubules, vacuolization (glycogen) and atrophy of some cells, and sclerosis of the basal membrane (Cardeza, Mancini, and Foglia, 1949-50). The blood pressure is normal, but diabetic rats develop hypertension following experimental perinephritis or treatment with desoxycorticosterone (desoxycortone, B.P.), with greater frequency and more rapidly than normal rats. They also have a larger volume of interstitial fluid (Braun-Menéndez and Martínez, 1949-50). Early on, the adrenals and kidneys become enlarged, while the thymus decreases (Foglia, 1945b).

In the first and second stages the administration of glucose provokes progressively higher hyperglycaemic curves with a greater formation of muscular and hepatic glycogen in relation to the intensity of hyperglycaemia. In the third stage the storage of glycogen, in particular in the liver, is much less for the same or greater degrees of hyperglycaemia (Foglia, 1945).

The incidence of diabetes and the rapidity of its development are in relation to the amount of pancreas removed, for which reason comparative experiments must be made using many controls, and preferably all the rats should be operated upon on the same day by the same skilled operator.

With the method of subtotal pancreatectomy the following problems may be studied: (a) the factors which raise the proportion of rats developing diabetes and increase its severity; (b) those which prevent or lower the incidence of its appearance; and (c) attempts at curative treatment of incipient or already confirmed diabetes.

Alloxanic and pancreatic diabetes are intensified by greater food intake (Martínez, 1946 ; Houssay and Martínez, 1947) ; hyperthyroidism (Houssay and Sara, 1945 ; Martínez, 1945-6 ; Houssay, Foglia, and Martínez, 1946 ; Houssay, 1937, 1946, 1948) ; diets rich in suet or lard (Martínez, 1945-6 ; Houssay and Martínez, 1947, 1948) ; and low-protein diet (Martínez, 1946 ; Houssay and Martínez, 1947).

*Ciba Foundation Lecture delivered on July 3.

Alloxanic and pancreatic diabetes are attenuated by thyroidectomy (Houssay, Foglia, Prieto Díaz, and Sara, 1945; Houssay and Sara, 1945; Martínez, 1945; Houssay, Foglia, and Martínez, 1946; Houssay, 1937, 1946, 1948) and by cysteine, the thiouracils, and other substances which increase the free SH groups in the tissues (Martínez, 1946; Houssay and Martínez, 1948; Houssay, 1950). The latter and coconut oil counteract the unfavourable effect of suet or lard (Houssay and Martínez, 1947, 1948). Certain substances such as thyroid and diethylstilboestrol at first increase the incidence and severity of diabetes, but when their action is continued there is later a decrease in both the incidence and the severity of the disease.

Difference in the Sex Incidence of Diabetes

A difference in the sex incidence of diabetes is evident when subtotal pancreatectomy (95%) has been carried out in male and female rats of the same age on the same diet *ad libitum*. The frequency is much higher in males

CHART 1.—Differences in the sex incidence of diabetes in 95% pancreatectomized rats. Left: with *ad libitum* feeding (93 females and 120 males). Right: paired feeding (16 pairs). (Foglia, Schuster, and Rodríguez, 1947.)

TABLE II.—Difference in the Sex Incidence of Diabetes in Subtotally Pancreatectomized Rats Six Months After Operation (Rodríguez, 1950a)

	No. of Rats Operated On	No. of Diabetics	Percentage
Male rats	140	125	89
Female rats	120	34	27

than in females (Foglia, 1945; Foglia, Schuster, and Rodríguez, 1947; Rodríguez, 1950a) (Chart 1; Table II).

The sexual difference is not due to different amounts of food. It is necessary to point this out, because in rats weighing 170 g. the males eat 27 g. and the females only 21 g. of the standard diet, and a greater food intake increases the incidence of diabetes while low food intake decreases it (Martínez, 1946; Houssay and Martínez, 1947).

In order to avoid such factors, experiments were made with paired feeding (Chart 1) and forced feeding (Chart 2). Rats of both sexes and the same weight were studied in pairs (Foglia, Schuster, and Rodríguez, 1947); the food intake of the female was weighed and the same amount given to the male the next day. In 16 paired-feeding experiments the incidence of diabetes was again higher in the males.

In forced-feeding experiments the rats received a constant quantity of food twice a day (Rodríguez, 1950a). After 40 days the incidence of diabetes in the males was

CHART 2.—Difference in the sex incidence of diabetes in 95% pancreatectomized rats forced-fed. (Rodríguez, 1950a.)

again much greater than in the females of the same weight.

These experiments with paired or forced feeding show that the difference in the sex incidence of diabetes in rats with subtotal pancreatectomy (95%) is not due to a difference in the amount of food ingested.

The sexual difference can be observed in the onset of diabetes which develops gradually after partial pancreatectomy. On the other hand, there is no sexual difference in the percentage of diabetic males and females in alloxanic diabetes (Rodríguez, 1950a) or in the severe diabetes of rats or dogs following total pancreatectomy.

Castration and Restitution

The sexual difference is due to a protective action of the ovary and a provocative action of the testicle, which is shown by experiments in castration and restitution.

The influence of castration in both sexes was shown by Foglia, Schuster, and Rodríguez (1947). In subtotal (95%) pancreatectomized rats removal of the testes diminishes the incidence of diabetes and removal of the ovaries increases it (Chart 3).

CHART 3.—Influence of castration on the incidence of diabetes after 95% pancreatectomy. Number of rats: 66 males; 32 castrated males; 30 females; 35 castrated females. (Foglia, Schuster, and Rodríguez, 1947.)

The incidence curves of male and female castrates are closer to each other than those of males and females with intact gonads. There is still, however, a considerable difference between them. These rats were castrated when their body weight was between 70 and 80 g. and had therefore been under the influence of sex hormones

before pancreatectomy was performed. Dr. Foglia is now studying the effects of castration performed immediately after birth.

The restitution of the ovaries by means of a graft at the same time as subtotal pancreatectomy was performed gave considerable protection. After five months only 44% (11 out of 25) of those which had an ovarian graft had diabetes, while 85% (13 out of 15) of the non-grafted castrates were diabetic.

Protection was greater in those rats in which the graft provoked more frequent and prolonged cycles (Foglia, 1949-50). Daily

CHART 4.—Influence of injection of oestradiol benzoate on the incidence of diabetes in rats after 95% pancreatectomy. Black columns: injected. White columns: controls. A, males; B, females; C, castrated males; D, castrated females. (Foglia, Schuster, and Rodríguez, 1947.)

subcutaneous injection of oestradiol during six months caused a marked decrease in the incidence of diabetes in castrates and controls of both sexes which had been submitted to subtotal (95%) pancreatectomy (Chart 4; Table III).

TABLE III.—Effect of Oestradiol Benzoate Injected Daily for Six Months on the Incidence of Diabetes in Intact and Castrated Male and Female Rats after Removal of 95% of the Pancreas (Foglia, Schuster, and Rodríguez, 1947)

Daily Dose per Rat (µg.)	Controls				Treated			
	Diabetic	%	Diabetic	%	Diabetic	%	Diabetic	%
Intact Males								
2	11/12	92	4/11	36	8/13	61	4/11	36
4	8/9	88	4/8	50	7/8	87	4/9	44
15	10/10	100	2/12	16	8/8	100	6/9	66
Castrated Males								
2	6/14	43	1/12	8	2/9	22	2/12	16
4	13/63	21	—	—	19/52	37	1/13	8
15	5/7	71	2/6	33	6/7	86	1/8	12
Intact Females								
2	6/14	43	1/12	8	2/9	22	2/12	16
4	13/63	21	—	—	19/52	37	1/13	8
15	5/7	71	2/6	33	6/7	86	1/8	12
Castrated Females								
2	6/14	43	1/12	8	2/9	22	2/12	16
4	13/63	21	—	—	19/52	37	1/13	8
15	5/7	71	2/6	33	6/7	86	1/8	12

Effects of Sex Hormones

In rats with subtotal pancreatectomy (95%) early treatment with oestrogens produced a biphasic effect: first the incidence and severity of diabetes increased, and later diabetes was attenuated or even definitely suppressed; initial intensification was seen, especially with diethylstilboestrol, and was well studied by Ingle. In forced-fed rats the appearance or intensification of glycosuria and hyperglycaemia has been observed: (a) in animals with partial pancreatectomy (Ingle, 1941; Rodríguez, 1950a); (b) temporarily in normal rats (Ingle, 1941); (c) in rats with alloxan diabetes (Houssay and Mazzocco, 1946; Ingle, Nezamis, and Prestrud, 1947; Ingle and Hogg, 1947); and (d) stilboestrol treatment provoked a fall in hyperglycaemia and glycosuria, but a high mortality, in rabbits with alloxan diabetes. Rats with alloxan diabetes fed *ad libitum* showed a fall in glycosuria and body weight, with an increase in liver glycogen, but no improvement in the diabetic state, when

submitted to oestrogen treatment (Janes and Dawson, 1946). The diabetogenic effect of oestrogens has also been observed in subtotally pancreatectomized adrenal-ectomized rats maintained with subdiabetogenic doses of cortical extract (Ingle, 1943), and in rats submitted to these operations but not given cortical extract (Rodríguez, 1950a). The intensity of the initial diabetogenic effect increases as the amount of remaining pancreatic tissue diminishes; it is also dependent on the dose of alloxan administered and the diet.

The following results have been observed in totally pancreatectomized animals treated with oestrogens; (a) attenuation of diabetes in dogs (Barnes and Regan, 1933; Barnes, Regan, and Nelson, 1933) and monkeys (Nelson and Overholser, 1936); (b) no effect in the monkey (Collip, Selye, and Neufeld, 1937) and dogs (Young, 1941); and (c) increase in severity of diabetes in the ferret (Dolin, Joseph, and Gaunt, 1941) and in rats (Martínez, unpublished results).

The protective effect of steroids against diabetes following subtotal pancreatectomy was demonstrated for oestradiol by Foglia, Schuster, and Rodríguez (1947) and then for several oestrogens (Chart 5, Table IV) by Lewis, Foglia, and Rodríguez (1949-50) and for phenocycline

CHART 5.—Incidence of diabetes in castrated female and castrated male rats, with subtotal pancreatectomy, injected daily for six months with oestrogens and androgens (controls with lactose); daily dose per rat in micrograms. (Lewis, Foglia, and Rodríguez, 1949-50.)

TABLE IV.—Effect of Different Substances Injected Daily for Six Months on the Incidence of Diabetes in Male and Female Castrated Rats after Removal of 95% of the Pancreas (Lewis, Foglia, and Rodríguez, 1949-50; Rodríguez, 1950a)

	Daily Dose per Rat (µg.)	Castrated Females				Castrated Males	
		Exp. A-D		Exp. B-C			
		Diabetics	%	Diabetics	%	Diabetics	%
Lactose	25 (mg.)	12/23	52	26/35	74	16/18	89
Cholesterol	500	4/15	27	5/9	55		
Oestrone	15	3/10	30				
	50	0/8	0				
Diethylstilboestrol	15	3/10	30				
	50			1/8	12	3/6	50
Phenocycline	15	0/9	0				
	50	1/11	9				
Dienoestrol				1/15	6		
Ethynil-oestradiol	50			4/16	25		
Testosterone	500			4/9	44		
Equilenine	50	6/8	75	6/9	66		
Progesterone	500			8/12	66		
Desoxycorticosterone	100						
Testosterone	50	11/15	73			13/14	92
	500	8/8	100				
Methyl-testosterone	500			9/11	83		
17-β-ethyl-dihydro-testosterone	500			8/11	72		

by Rodríguez (1950b). Androgens, on the contrary, caused an earlier appearance and increased the incidence and severity of diabetes.

The different substances were injected daily during six months after removal of 95% of the pancreas. The incidence of diabetes was decreased in castrated females by treatment with the following substances: oestron, oestradiol, stilboestrol, dienioestrol, phenocycline, ethynil-oestradiol, and ethynil-testosterone. The incidence of diabetes also decreased in male castrates treated with oestrone and stilboestrol, which were the only oestrogens tested in these animals. Androgens (testosterone and methyl-testosterone) markedly increased the incidence and severity of diabetes in male and female castrates. The incidence and course of diabetes were not modified by treatment with progesterone, desoxycorticosterone, and 17- β -ethyl-dihydro-testosterone; equilenine was also inactive in the doses tested. Cholesterol produced a slight but definite decrease in the incidence of diabetes, although it has no oestrogenic effect.

To sum up, substances with oestrogenic effect decreased the incidence of diabetes in white rats following subtotal pancreatectomy. Androgens, on the contrary, increased it and other steroids had no effect.

In most animals treated with steroids the increase in body weight was less than in the controls. Many of the rats treated with oestrogens showed a loss of hair, which was considerable in some cases, while the coats of rats treated with androgens became coarse. Animals treated with oestrogens showed an increase in the weight of the hypophysis, adrenals, and uterus, and hypertrophy and hyperplasia of the islets of Langerhans in the remaining pancreatic tissue. Animals treated with testosterone had small adrenals and hypophysis. The weight of the liver and kidneys increased in animals treated with oestrogens and androgens (Lewis, Foglia, and Rodríguez, 1949-50).

Mechanism of Oestrogen Action

In order to see whether the protective effect of oestrogens was due to a difference in the amount of food ingested by the rats submitted to treatment, a series of subtotally pancreatectomized animals were forced-fed, and half of them were injected with 50 μ g. of diethylstilboestrol daily. During the first month diabetes appeared in the treated animals, then the blood sugar fell to a normal level and glycosuria disappeared.

CHART 6.—Variation of the glycaemia of castrated and partially pancreatectomized female rats under forced feeding, injected with 50 μ g. of diethylstilboestrol daily, and controls. (Rodríguez, 1950c.)

CHART 7.—Variation of the glycosuria of castrated and partially pancreatectomized female rats under forced feeding, injected with 50 μ g. of diethylstilboestrol daily, and controls (average of five injected rats and seven controls). (Rodríguez, 1950c.)

In the forced-fed controls the blood sugar was at first normal, but between the 45th and 75th days after operation they all became diabetic. This experiment clearly demonstrates the initial diabetogenic and subsequent protective effect of stilboestrol (Rodríguez, 1950c).

Substances which exert a protective action against diabetes enlarge the hypophysis in most cases, but no strict correlation was observed between the increase in hypophysial weight and the protective effect (Lewis, Foglia, and Rodríguez, 1949-50).

The administration of oestrogens in rats increased the weight of the adrenals. However, the protective action could not be attributed to the adrenals, as it occurred in subtotally pancreatectomized rats already diabetic which were then adrenalectomized. Diethylstilboestrol produced first its slight diabetogenic action, and later the diabetes disappeared definitely in four out of seven rats (Chart 8). In the untreated rats adrenalectomy produced a transient decrease in the blood sugar, but after four to six weeks all the animals became diabetic again as the accessory adrenals hypertrophied; when this occurred those treated with oestradiol were protected.

The protective action of oestrogens seems to be due chiefly to the fact that they produce hypertrophy and hyperplasia of the islets of Langerhans in the pancreas. This protective action is observed in the diabetes resulting from subtotal pancreatectomy (Lewis, Foglia, and Rodríguez, 1949-50), but not in that resulting from total or almost total pancreatectomy.

Subtotal (95%) pancreatectomy is followed by a compensatory hypertrophy of the islets of Langerhans accompanied by sclerosis in some parts. This hypertrophy may be considerable after a year or more if the animal has not become diabetic. When diabetes occurs the β -cells are damaged and progressively disappear and the islets atrophy. If the diabetes

CHART 8.—Variation of the glycaemia of adrenalectomized and partially pancreatectomized male rats, under forced feeding, injected daily with 50 µg. of diethylstilboestrol, and controls. (Rodríguez, 1950a.)

occurs early there is only atrophy, but if it appears later there is first hypertrophy and then atrophy.

It has been noticed that the female rat has a greater volume of islets than the male (Tejning, 1947). After subtotal pancreatectomy the incidence of diabetes is less in females than in males, but, on the other hand, females more often present hypertrophy and hyperplasia of the islets of Langerhans (Lewis, Foglia, and Rodríguez, 1949–50; Cardeza, 1950). The stimulating factor in the female is the ovarian hormone (Table V).

TABLE V.—Ratio Between Pancreatic Islets and Acini of Non-diabetic Castrated Female Rats, with Subtotal Pancreatectomy, Injected with Steroids During Six Months. Controls with Lactose. (Cardeza, 1950)

Substance	No. of Animals	Weight Islets × 100 Weight Acini
Lactose	4	1.66
Ethinyl-oestradiol	2	6.94
Diethylstilboestrol	4	6.93
Oestrone	2	6.77
Dienoestrol	2	6.18
Oestradiol	3	5.42
Ethinyl-testosterone	1	2.98
Progesterone	1	1.84

Oestrogens rapidly produce hypertrophy of the islets, with the formation of new islets (Del Castillo and Sammartino, 1937; Lewis, Foglia, and Rodríguez, 1949–50; Cardeza, 1950).

Confluent masses of newly formed islets can be observed which have a multilobular or ramified appearance. In certain cases the newly formed islets occupy a whole lobule of the pancreas, displacing the acini and giving the appearance of an insular adenoma (Plate, Figs. 1, 2, and 3). Hyperplasia and increase of centro-acinar cells is widespread, with the development of cells similar to β -cells (Cardeza, 1950; Rodríguez, 1950d) (Plate, Figs. 4, 5, 6, and 7). On the contrary, rats treated with androgens show early diabetes with atrophy of the islets and a marked decrease in β -cells.

Oestrogens appear to produce a direct action on the insular tissue. After intrapancreatic implantation of oestradiol, normal rats, guinea-pigs, and cats showed at first hyperplasia of the centro-acinar cells (Plate, Fig. 8), then newly formed islets containing hypertrophied cells of the β type (Plate, Fig. 9). The formation of new islet tissue was more pronounced in the zone of oestrogenic implantation.

There are two principal groups of substances which can prevent the appearance of diabetes in subtotally

pancreatectomized rats—that is, oestrogens and substances which increase the free SH groups in the tissues (cysteine, thiouracils, etc.). Rodríguez and Martínez (1950) treated groups of subtotally pancreatectomized castrated female rats with oestradiol or thiouracil, or both together. Treatment with oestradiol produced two phases: an initial increase of the diabetes, followed by later protection which persisted after the treatment was discontinued. Thiouracil prevented the appearance of diabetes during treatment, but diabetes reappeared soon after treatment was discontinued in all cases.

Administration of both substances together afforded a greater degree of protection than when given separately, but on withdrawal diabetes reappeared in many animals; only 25% were protected when oestradiol alone was used.

This difference between the two substances is due to the fact that rats which received propylthiouracil were protected by its metabolic action, but it was not accompanied by hypertrophy and hyperplasia of the islets. On the other hand, rats treated with oestradiol had hypertrophy and hyperplasia of the islets of Langerhans, to which apparently the lasting protection against diabetes is due.

In some cases of alloxan diabetes a curative action of oestradiol associated with insulin has been found (Table VI). Experiments are being repeated on a larger number of animals.

TABLE VI.—Rats with Alloxan Diabetes Treated for Six Months

Treatment	Cured	Diabetic	Dead
Oestradiol and insulin	4	1	3
Insulin	0	3	5
Oestradiol	0	2	6

Summary and Conclusions

This group of experiments shows that after subtotal pancreatectomy diabetes appears in rats with less frequency in the female than the male. This sexual difference is due to a provocative action of the testes and androgens and a protecting influence of the ovaries and oestrogens.

The mechanism of protection is apparently chiefly due to the action of the ovaries and oestrogens in the stimulation of hypertrophy and hyperplasia of the islets of Langerhans with production of new β -cells at the expense of the centro-acinar cells.

It is difficult to say yet how far this action occurs in other species, and I would rather not discuss now whether or not it occurs in man.

The peripheral action of oestrogens upon carbohydrate metabolism is not yet well known.

The probability of preventing some forms of experimental diabetes, and even of curing a certain proportion of cases of not too severe diabetes, certainly exists.

It is evidently worth while to continue this line of investigation—using prolonged administration of those substances already studied and other new ones, either alone or combined with insulin—in order to prevent or treat other types of experimental diabetes, and eventually human diabetes.

It would be of value to discover substances which have no oestrogenic effect but cause hyperplasia of the islets and inhibit the hypophysis or adrenals. Diabetes can possibly

be controlled by other mechanisms, such as direct or indirect action on tissue metabolism.

Experimental medicine has given us most of our fundamental knowledge of diabetes, and it seems that it will continue to increase this knowledge. It also gives us hope that new methods that will prevent or even cure this disease may be discovered.

BIBLIOGRAPHY

- Barnes, B. O., and Regan, J. F. (1933). *Endocrinology*, **17**, 522.
— and Nelson, W. O. (1933). *J. Amer. med. Ass.*, **101**, 926.
Braun-Menéndez, E., and Martínez, C. (1949-50). *Rev. Soc. argent. Biol.*, **25**, 162, 168; *C.R. Soc. Biol., Paris*, **144**, 415, 417.
Cardeza, A. F. (1950). *Rev. Soc. argent. Biol.*, **26**, 150.
— Mancini, R. E., and Foglia, V. G. (1949-50). *Rev. Soc. argent. Biol.*, **25**, 278; *C.R. Soc. Biol., Paris*, **144**, 425.
— and Rodríguez, R. R. (1949-50). *Rev. Soc. argent. Biol.*, **25**, 178; *C.R. Soc. Biol., Paris*, **144**, 421.
Collip, J. B., Selye, H., and Neufeld, A. (1937). *Amer. J. Physiol.*, **119**, 289.
Del Castillo, E. B., and Sammartino, R. (1937). *Rev. Soc. argent. Biol.*, **13**, 455.
Dolin, G., Joseph, S., and Gaunt, R. (1941). *Endocrinology*, **28**, 840.
Foglia, V. G. (1944). *Rev. Soc. argent. Biol.*, **20**, 21.
— (1945a). *Ibid.*, **21**, 7.
— (1945b). *Ibid.*, **21**, 45.
— (1945c). *Ibid.*, **21**, 360.
— (1949-50). *Ibid.*, **25**, 250; *C.R. Soc. Biol., Paris*, **144**, 424.
— and Cramer, F. K. (1944). *Proc. Soc. exp. Biol., N.Y.*, **55**, 218.
— Mancini, R. E., and Cardeza, A. F. (1950). *Arch. Path.*, **50**, 75.
— Orias, O., and Sara, J. G. (1944). *Rev. Soc. argent. Biol.*, **20**, 440.
— Schuster, N., and Rodríguez, R. R. (1947). *Endocrinology*, **41**, 428; *Rev. Soc. argent. Biol.*, **23**, 202.
Houssay, B. A. (1937). *Amer. J. med. Sci.*, **193**, 581.
— (1946). *Vitamins and Hormones*, **4**, 187.
— (1948). *Recent Progr. Hormon. Res.*, **2**, 277.
— (1950). *Amer. J. ment. Sci.*, **219**, 353.
— Foglia, V. G., and Martínez, C. (1946). *Endocrinology*, **39**, 361.
— Prieto Díaz, H., and Sara, J. G. (1945). *Rev. Soc. argent. Biol.*, **21**, 232.
— Lott, W. A., and Martínez, C. (1950). *Ibid.*, **26**, 335.
— and Martínez, C. (1947). *Science*, **105**, 548.
— (1948). *Rev. Soc. argent. Biol.*, **24**, 63, 123, 242; *C.R. Soc. Biol., Paris*, **142**, 1167, 1573.
— and Mazzocco, P. (1946). *Rev. Soc. argent. Biol.*, **22**, 367.
— and Sara, J. G. (1945). *Ibid.*, **21**, 81.
Ingle, D. J. (1941). *Endocrinology*, **29**, 838.
— (1943). *Amer. J. Physiol.*, **138**, 577.
— and Hogg, J. A. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 244.
— Nezamis, J. E., and Prestrud, M. C. (1947). *Endocrinology*, **41**, 207.
Janes, R. G., and Dawson, H. (1946). *Ibid.*, **38**, 10.
Lewis, J. T., Foglia, V. G., and Rodríguez, R. R. (1949-50). *Rev. Soc. argent. Biol.*, **25**, 67; *Endocrinology*, **46**, 111.
Martínez, C. (1945a). *Rev. Soc. argent. Biol.*, **21**, 254.
— (1945b). *Ibid.*, **21**, 332.
— (1946a). *Ibid.*, **22**, 414.
— (1946b). *Ibid.*, **22**, 135.
— (1946c). *Ibid.*, **22**, 428.
Mering, J. von, and Minkowski, O. (1889). *Versamml. Dtsch. Natur. Aerzte, Heidelberg*, **23**, 393.
Nelson, W. O., and Overholser, M. D. (1936). *Endocrinology*, **20**, 473.
Rodríguez, R. R. (1950a). *Rev. Soc. argent. Biol.*, **26**, 205.
— (1950b). *Ibid.*, **26**, 247.
— (1950c). *Proc. Soc. exp. Biol., N.Y.*, **73**, 317.
— (1950d). Tesis Fac. Med. B. Aires.
— and Martínez, C. (1950). *Rev. Soc. argent. Biol.*, **26**, 127.
Shapiro, R., and Pincus, G. (1936). *Proc. Soc. exp. Biol., N.Y.*, **34**, 416.
Souto-Maior, M. G., and Foglia, V. G. (1944). *Rev. Soc. argent. Biol.*, **20**, 55.
Tejning, S. (1947). *Acta med. scand.*, Suppl. 198.
Young, F. G. (1941). *Lancet*, **1**, 600.

A welfare worker of the Save the Children Fund has been working in Northern Papua since mid-May as part of the disaster relief organized in New Guinea after the volcanic explosion there early this year (*The World's Children*, September, 1951). She remarks that the average Papuan infant is smaller than the European at birth, weighing about 5 lb. Weaning begins at about 6 months old, with hard indigestible taro and rice, but an attempt is being made to teach the mothers cooking of vegetables and the preparation of porridge and other types of infant food.

ORAL TREATMENT OF POLYCYTHAEMIA VERA WITH β -NAPHTHYL-DI-2-CHLOROETHYLAMINE (R48)

BY

KURT IVERSEN, M.D.

AND

E. MEULENGRACHT, M.D.

(From Medical Department B, Bispebjerg Hospital,
Copenhagen)

Since Gilman and Philips (1946) and Rhoads (1946) published the first communications on the therapeutic action of nitrogen mustards (methyl bis- β -chloroethylamine and methyl tri- β -chloroethylamine), a number of reports on the use of these substances have appeared. On the whole there seems to be agreement that, given intravenously, nitrogen mustards can produce a distinct, but only temporary, improvement in some cases of malignant lymphogranulomatosis, lymphosarcoma, chronic leukaemia, and polycythaemia vera. As is known, the substances are cell poisons, and their inhibiting effect on bone marrow involves some risk. Thus cases of agranulocytosis have been met with after intravenous treatment with nitrogen mustard (Bjurwill, 1947; Iversen, 1951). There are also other drawbacks, such as the tendency to the formation of thrombi at the site of injection, and nausea and vomiting following the injection, while the intravenous method itself is a disadvantage, particularly as it must be carried out with due observance of various precautionary measures.

R48 by Oral Administration

Continuous work is being done with these fairly simple substances from a chemical standpoint, in order if possible to discover some which act more selectively and are less toxic. For instance, Burchenal (1948) tested two kinds of complex aliphatic nitrogen mustards, but they proved to be too toxic.

Haddow and others (1948) prepared a series of aromatic nitrogen mustards, the action of which they tested on experimentally produced tumours. Some of these substances turned out to be unsuitable for clinical use owing to their toxic effects. One of them, β -naphthyl-di-2-chloroethylamine (R48), however, seemed quite appropriate for a clinical trial. It had the added advantage that it could be administered orally. Clinical investigations were carried out in Oxford, and Matthews (1950) has published a preliminary report on the findings in 17 patients with various grave blood diseases. He found some improvement in five cases of Hodgkin's disease, but not more than has been observed to follow treatment with methyl-bis- β -chloroethylamine. At any rate R48 was unsuitable for the treatment of patients with localized masses of glands; x-ray treatment produced far better results. In two cases of reticulosarcoma and two cases of acute leukaemia there was no effect. In two out of four cases of chronic myeloid leukaemia a surprisingly good remission occurred which lasted for 11-28 weeks. These remissions could be reproduced without toxic effects. But the observation period for all these patients is still too short. The best result was observed in a patient with polycythaemia vera

B. A. HOUSSAY: SEX HORMONES AND DIABETES

FIG. 1.—Pancreas of rat treated with lactose (controls). Moderate hyperplasia of islets; peri-insular sclerosis. (Cardeza, 1950.)

FIG. 2.—Pancreas of rat treated with diethylstilboestrol. Intense hyperplasia, adenomatous. (Cardeza, 1950.)

FIG. 3.—Pancreas of rat treated with ethinyl-oestradiol. Diffuse hyperplasia of islets. (Cardeza, 1950.)

FIG. 4.

FIG. 6.

FIG. 7.

FIG. 5.

FIGS. 4-7.—Pancreas of rats treated with diethylstilboestrol, FIG. 4: Adenomatous hyperplasia of insular cells. FIG. 5: Hyperplasia of centro-acinar cells. FIG. 6: Newly formed islets from centro-acinar cells. FIG. 7: Mixed formation acino-insular. (Cardeza, 1950.)

FIG. 8.

FIG. 9.

FIGS. 8 AND 9.—Local action of oestradiol on the islets of Langerhans of the cat. FIG. 8:—In H: hyperplasia of centro-acinar cells with granulation. FIG. 9: Newly formed centro-acinar cells with granulation. (Cardeza and Rodríguez, 1949-50.)