anaemia, but he had to be removed to another hospital for treatment, because never in the history of the first hospital had a blood transfusion been given.

Summary

Twenty-four cases of aplastic anaemia caused by the absorption of trinitrotoluene (15 being fatal) are recorded. Of the nine patients who recovered, six were treated by multiple blood transfusions with administration of liver, iron, and vitamins; three received liver, iron, and vitamin therapy, but no blood transfusion; one of these three also received ventriculin.

Analysis is made of these 24 cases and of three cases of early toxic anaemia due to the same cause. The insidious onset of aplastic anaemia sets a difficult problem.

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An interim statement has been made to the Minister of Health, Mr. E. A. NUGAWELA, by Mr. DONALD BARLOW and Professor ROBERT NEUBAUER, of the World Health Organization, and Professor JOHN CROFTON, of Edinburgh, on the progress made by the Ceylon Government in implementing Mr. Barlow's recommendations, made in 1952, for reorganizing the campaign against tuberculosis. On returning to Ceylon, states the report, Mr. Barlow was forcibly struck by the many improvements which had occurred. Great strides had been made in both prevention and cure. The team felt that there was still a long way to go, but the Government had attacked the problem with vigour and determination. No Government could have been more alert to its responsibilities. The report draws attention to the enthusiasm of those taking part in the scheme, noting that the hopelessness on the faces of the patients in the grossly overcrowded and ill-equipped wards had given place to hope, cheerfulness, and expectation of recovery. X-ray facilities are being increased, and a start has been made in the provision of pathological and bacteriological services. Since 1952 the mortality from diagnosed tuberculosis has been halved. This is attributed partly to the provision by the Government of large funds for the purchase of modern drugs. The training of doctors and nurses still presents a big problem. A fair number have taken advantage of the Colombo Plan Scheme of training in England, but at least one more fully staffed and equipped medical school is required in Ceylon.

THE EFFECT OF INSULIN ON NITROGEN RETENTION IN THE HYPOPHYSECTOMIZED RAT

BY

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It has been reported from this laboratory that exogenous insulin promotes growth in hypophysectomized rats.^{1,2} These animals gain weight owing to increases in protein, fat, and water. The observed increases in body protein stimulated us to study in some detail the effect of insulin on nitrogen retention.

Procedure

Male rats with an average body weight of 119 g. which had been hypophysectomized 14 days previously were used in all experiments. The animals were maintained in a constant-temperature room at 28° C. In Experiment A the daily nitrogen balances of six untreated hypophysectomized rats were determined for a 12-day period. In Experiment B hypophysectomized rats were divided into one control group of 12 animals and one experimental group of eight animals. Nitrogen-balance studies were not performed on the controls, but a good indication of the balance maintained by these animals during the experimental period was secured by determining changes in the nitrogen content of their carcasses. This observation was made by sacrificing six of the rats on the initial day of the experiment and comparing their average total body nitrogen with a similar value obtained from the remaining six controls sacrificed on the last day of the experiment.

The daily nitrogen balance of each of the eight rats in the experimental group was determined for (a) an initial period of three days before insulin was administered; (b) an intermediate period of seven days during which each rat received one daily injection of increasing amounts of protamine zinc insulin; and (c) a four-day period following the cessation of insulin treatment. These rats were sacrificed with surviving members of the control group on the fourteenth day. Total body nitrogen determinations were made on each carcass. The nitrogen balance was regarded as the difference between nitrogen ingested and nitrogen excreted in the faeces and urine.

The food mixture consisted of the following ingredients: water, 1,200 ml.; "Chum" dog food, 890 g.; dried white bread crumbs, 335 g.; powdered skim-milk, 145 g. The glucose was dissolved in the water and this solution used to reconstitute the powdered milk. The remaining constituents were thoroughly mixed in and the whole was homogenized in a Waring blender.

Dry-weight determinations were made on nine samples of the diet. The nitrogen contents of these dried samples were determined. The results agreed very closely and showed that the diet was well homogenized.

All rats ate *ad libitum*. To avoid errors due to evaporation, the amount of food given each rat daily was calculated in terms of its dry weight. The food remaining from the previous day was removed and dried to constant weight. The dry weight of the food eaten was then determined and

the nitrogen intake calculated. Errors in estimations of nitrogen intake were minimized by eliminating rats that showed a tendency to scatter their food.

Each rat was placed in a cylindrical wire metabolism cage suspended over a large funnel. A fine wire mesh prevented the faeces from passing into the funnel below. The stools were carefully collected from the bottom of the cage once daily. Each collection of stools was digested in sulphuric acid with a selenium catalyst. The digest was then diluted and nitrogen determinations were made on aliquots.

A thin layer of glass wool placed in the apex of the funnel prevented hair and scales from passing into the vessel in which the urine was collected under toluene. The cage floor, funnel, and glass wool were washed down thoroughly with distilled water at each urine collection. The total volume of the combined urine and washings was measured and an aliquot retained for nitrogen determinations.

Total body fat was determined on the carcasses of animals used in this investigation in connexion with other work. Therefore the total body nitrogen content of each rat was determined from the fat-free residue in the following way. The whole defatted carcass was digested for 10 hours in boiling sulphuric acid containing selenium catalyst mixture. The digest was then diluted and aliquots of this

were redigested before their nitrogen contents were determined. All nitrogen determinations were carried out in duplicate by the micro-Kjeldahl technique.

Results

The results of this investigation are presented in Tables I, II, and III and the Chart. It was found in Experiment A that under the conditions of this investigation untreated hypophysectomized rats remain in nitrogen equilibrium (Table I and Chart). This observation was supported by the results of Experiment B. The untreated controls remained in nitrogen equilibrium, since their average total body nitrogen content did not change during the experimental period (Table III). The carcass analysis shows that the nitrogen content of the insulin-treated hypophysectomized rats was greatly increased (Table III). This is in accord with the data obtained from the nitrogen-balance studies carried out on these animals. When insulin was administered to hypophysectomized rats (Chart) there

TABLE I.—Average Daily Nitrogen Balance of Six Untreated Hypophysectomized Rats

Day	Av. Wt. (g.)	Food Mg.-N ₂	Urine Mg.-N ₂	Faeces Mg.-N ₂	Balance Mg.-N ₂	% N ₂ Re-tained	% N ₂ Urine	% N ₂ Faeces
1	119.0	205.1	174.3	28.8	+2.0	0.97	84.98	14.04
2	118.8	209.0	175.3	29.7	+4.0	1.91	83.87	14.21
3	119.1	205.1	172.5	26.9	+5.7	2.78	84.10	13.12
4	118.8	210.1	185.6	29.3	-4.8	-2.28	88.33	13.95
5	119.1	206.5	176.0	27.7	+2.8	1.36	85.23	13.41
6	119.7	199.6	175.3	26.8	-2.5	-1.25	87.82	13.43
7	119.6	205.5	172.1	30.0	+3.4	1.65	83.74	14.60
8	119.6	204.3	172.0	29.3	+3.0	1.47	84.19	14.34
9	120.0	206.8	177.8	29.3	-0.3	-0.15	85.97	14.17
10	120.2	203.0	170.6	28.6	+3.8	1.87	84.03	14.09
11	120.8	204.6	173.5	28.1	+3.0	1.47	84.79	13.73
12	121.0	204.3	172.8	28.5	+3.0	1.47	84.58	13.95

TABLE II.—Average Daily Nitrogen Balance of Eight Insulin-treated Hypophysectomized Rats

Day	Insulin Units	Food Mg.-N ₂	Urine Mg.-N ₂	Faeces Mg.-N ₂	Balance Mg.-N ₂	% N ₂ Re-tained	% N ₂ Urine	% N ₂ Faeces
1	0	169.2	143.9	18.0	+7.3	+4.31	85.05	10.64
2	0	212.6	170.8	35.9	+5.9	+2.77	80.34	16.89
3	0	197.2	175.4	29.3	-7.5	-3.80	88.95	14.86
4	0.5	211.2	163.8	37.3	+10.1	4.78	77.56	17.66
5	1.0	240.8	187.9	36.2	+16.7	6.94	78.03	15.03
6	1.0	228.1	149.9	34.0	+44.2	19.38	65.71	14.91
7	1.4	249.3	133.5	39.6	+76.2	30.56	53.55	15.88
8	1.8	268.6	152.2	36.6	+79.8	29.70	56.66	13.63
9	2.4	262.7	136.6	38.8	+87.3	33.23	52.00	14.77
10	3.0	280.4	130.9	39.9	+109.6	39.09	46.68	14.23
11	0	203.4	110.1	40.1	+53.2	26.15	54.13	19.71
12	0	180.0	131.5	33.5	+15.0	+8.33	73.05	18.61
13	0	181.6	172.0	33.1	-23.5	-12.94	94.71	18.23
14	0	190.2	154.8	25.6	+9.8	5.15	81.39	11.46

Changes in the average nitrogen intake, nitrogen retention, and body weight of six untreated hypophysectomized rats and eight insulin-treated hypophysectomized rats. The dose of insulin was 0.5 unit for the first day and 1, 1, 1.4, 1.8, 2.4, and 3 units for the subsequent days.

TABLE III.—Total Body Nitrogen Contents of Hypophysectomized Rats

Controls Killed Day 1				Controls Killed Day 14				Insulin-treated Killed Day 14			
Rat No.	Final Fasting Wt. (g.)	Wt. Change (g.)	Total Body N ₂ (mg.)	Rat No.	Final Fasting Wt. (g.)	Wt. Change (g.)	Total Body N ₂ (mg.)	Rat No.	Final Fasting Wt. (g.)	Wt.* Change (g.)	Total Body N ₂ (mg.)
9	110.9	0	3,235	15	115.0	4.0	3,204	1	127.6	9.0	3,829
10	117.4	0	3,316	16	118.2	1.5	3,288	2	113.0	7.9	3,602
11	106.2	0	3,186	17	117.3	5.8	3,250	3	118.9	1.1	3,683
12	114.2	0	3,282	18	121.0	4.0	3,300	4	126.8	9.8	3,950
13	118.0	0	3,344	19	132.0	4.9	3,461	5	118.0	7.2	3,800
14	123.3	0	3,475	20	125.9	6.9	3,365	6	118.0	1.9	3,807
								7	117.5	4.1	3,737
								8	109.8	-3.0	3,713
Average	115		3,306		121.6	4.5	3,311		118.7	4.7	3,765

* During the last four days of the experiment rats in this group lost much of the weight they had gained (see Chart and Discussion).

was a marked increase in the absolute amount and percentage of nitrogen retained. The increase in food intake and in body weight is apparent. The changes in the percentage of nitrogen retained were due to changes in the proportion of ingested nitrogen excreted in the urine (Table II), whereas the percentage of the ingested nitrogen that was excreted in the faeces remained relatively constant. It is apparent (Chart) that nitrogen equilibrium was restored when the insulin injections were stopped. This latter change in nitrogen balance was accompanied by a fall in body weight. The loss of weight was due to the loss of fat or water or of both. No appreciable amount of protein was lost, since the animals did not exhibit a significantly negative nitrogen balance.

Discussion

The hypophysectomized insulin-treated rats were not maintained on a restricted diet, since this preparation is extremely sensitive to the hypoglycaemic action of insulin. The effect of the increased caloric intake has therefore not been defined. MacKay *et al.*³ have shown that insulin enhances nitrogen retention in intact rats maintained on a restricted diet.

Our data suggest that a linear relationship may exist between insulin dosage and nitrogen retention. This relationship might have been more apparent if it had been possible to prevent compensatory decreases in pancreatic beta-cell activity which undoubtedly occurred. Chaikoff and Forker⁴ have shown that in depancreatized dogs the amount of insulin administered is linearly related to the amount of nitrogen retained.

Since Banting, Campbell, and Fletcher⁵ first showed that insulin decreases the nitrogen excreted by diabetic patients, abundant evidence has accumulated to indicate that insulin is intimately associated with protein synthesis. It has been shown *in vivo* and *in vitro* that the antidiabetic hormone stimulates the utilization of amino-acids and the synthesis of proteins and inhibits protein catabolism.^{6,16} The results of our experiments are in accord with this evidence and they confirm and extend our previous reports.

Summary

The effect of insulin on nitrogen balance in the hypophysectomized rat has been studied. It has been found that under the conditions of this investigation hypophysectomized rats treated with insulin show a marked increase in the absolute amount and in the percentage of nitrogen retained. Over the range studied the percentage of ingested nitrogen retained bore an approximately linear relationship to the amount of insulin given.

The results of these experiments confirm and extend the earlier reports from this laboratory that insulin can function as a growth hormone in the absence of pituitary factors.

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THE USE AND ACTION OF CHLORPROMAZINE IN PSYCHONEUROSES

BY

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Of recent years a number of compounds with specific pharmacodynamic properties have been developed in France from derivatives of the aromatic base phenothiazine. These compounds include promethazine hydrochloride ("phenergan"), diethazine hydrochloride ("diparcol"), ethopropazine hydrochloride ("lysivane"), and promethazine 8-chlorotheophyllinate ("avomine"). Their usefulness lies in their specific antagonism towards naturally occurring histamine, adrenaline, and acetylcholine, and in the central depressant action which they show in varying degrees, the exact mechanism of which is not clearly understood.

A new derivative of phenothiazine—chlorpromazine hydrochloride (3-chloro-10-(3'-dimethylaminopropyl)-phenothiazine; "largactil")—is now available in this country. Like the others, it was developed in France, and clinical trials there indicated its possibilities in anaesthesia, as an anti-emetic agent, and in the treatment of psychiatric illness.

In the psychiatric field, Sigwald and Bouttier (1953a) claimed that the drug exerted a depressant action on the central and autonomic nervous systems, and that it displayed special properties which they have called "neuroplegic," producing results comparable to those of "frontal lobotomy." The drug has been employed in the treatment of both psychotic and psychoneurotic patients. Sigwald and Bouttier (1953b) published the results in a series of 48 patients, of whom 12 appear to have been cases of psychosis, while the remainder were psychoneurotic.

These investigators state that chlorpromazine nearly always improved the mood of their patients, and led to the disappearance of depression, with the frequent development of some degree of euphoria. Patients are said to have become indifferent to their worries. Anxiety was replaced by a feeling of calm and detachment, and morbid thoughts disappeared. They state furthermore that "the effect on obsessions is often significant, and sometimes remarkable," while there is "a rapid and complete alleviation of fears, scruples, doubts, and phobias." True obsessional states, on the other hand, are said to give poorer results, though the patient is able to overcome his obsessional thoughts more easily.

The results of treatment with chlorpromazine are said to be characterized by a significant percentage of successes, and analysis of their results shows that these were "good" or "very good" in 50 to 60% of each of the psychoneurotic manifestations they investigated.

In view of the encouraging possibilities of chlorpromazine over such a wide field, it was decided to investigate its effect on a series of psychoneurotics comparable to that of Sigwald and Bouttier.