Table.—Influence of Varying Amount of Activated Sludge on the Removal of Phosphorus from Sewage*

Amount of sludge** (percentage) added to sewage and aerated for 6 h	Percentage reduction of turbidity***	Percentage purification based on 3-min permanganate value	Percentage removal of	
			Water soluble Phosphorus (P)	Total Phosphorus (P)
Raw sewage only (control)	4·5	6·9	2·1	1·9
2·5	18·2	22·8	21·7	11·3
5·0	27·3	38·6	26·1	17·7
7·5	40·9	62·8	32·6	40·4
10·0	59·1	78·7	43·5	53·5
15·0	72·8	83·5	82·6	78·1
20·0	77·3	88·3	91·3	90·5
25·0	86·4	88·3	93·2	92·6
30·0	90·9	88·3	94·2	93·6

* The analytical figures for the sewage employed are: turbidity 125; 3-min permanganate value 22 p.p.m.; water-soluble phosphorus (P) 7·7 p.p.m. and total phosphorus (P) 10·3 p.p.m.

** Microscopic examination of the sludge showed the presence of certain protozoa in large numbers, notably the species of *Opercularia* (about 3000 cells per ml) and *Epistylis* (about 800 cells per ml).

*** Turbidity was determined with the aid of a Klett Summerson photoelectric colorimeter, employing violet filter (420 mμ).

The results given in the Figure and the Table show that during the activated sludge process the removal of phosphorus, including the water-soluble phosphorus, from the sewage closely follows the rapid rate of clarification and oxidation of sewage and that the rapidity of removal of the phosphorus, as the purification process, is dependent on the concentration of sludge.

Other observations may be summarised as follows:

When the sludge was previously heated at temperatures above 40°C for 10 min and added to sewage, the removal of phosphorus, as the purification of sewage, was adversely affected. The sludge heated at 50°C for 10 min did not remove the water-soluble phosphorus and did not purify the sewage to any appreciable extent. Treatment of the mixture of sewage and sludge with small amounts of chemicals such as mercuric chloride (4 p.p.m.) also adversely affected both the removal of phosphorus and the purification process. Addition of a mixed culture of bacteria isolated from activated sludge (15 ml bacterial suspension, each ml containing 8000 millions of bacteria, added to 85 ml of raw sewage; the bacteria free from the culture medium were obtained by the technique[5] using cellophane membrane on nutrient agar) caused only a slight reduction of water-soluble phosphorus but caused an appreciable increase in the total phosphorus, in the permanganate value, and in turbidity, these increases being due to the added suspension of bacteria.

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Résumé

La plus grande partie du phosphore contenu dans les eaux d'égouts y compris le phosphore dissous dans l'eau a été rapidement éliminée à l'aide de boue «activée», en l'espace de 6 h. Le liquide recueilli par le procédé de boue «activée» contient très peu de phosphore. Le taux du phosphore éliminé suit de très près la vitesse de purification et d'oxydation des eaux d'égouts. L'activité des bactéries contenues dans la vase ne semble pas expliquer la disparition rapide du phosphore contenu dans les eaux d'égouts.

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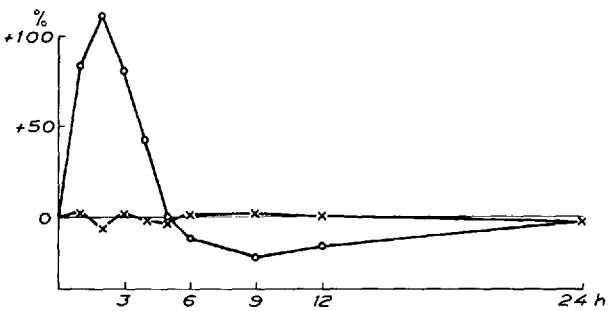
Effect of Cobaltous Chloride on the Blood Sugar Level and the Islet Cells in Rats

Since 1951, when VAN CAMPENHOUT and CORNELIS[1] demonstrated changes in the α-cells of the islets of Langerhans in guinea-pigs after injection of cobaltous chloride, similar observations have been made in other species of animals such as the rabbit and the dog[2–4]. Disturbance of the blood sugar level has been reported in a number of cases, the change usually consisting in an increase instead of the decrease that might logically be expected. CREUTZFELDT and SCHMIDT[5] and FODDEN[6] have not been able to observe such changes in the blood sugar or islet cells in rats. MUKHERJEE, DE and MUKERJI[7], on the other hand, obtained hyperglycemia after cobaltous chloride administration (50 mg/kg), but these investigators did not study the islets from the morphological aspect.



Blood sugar changes during 24 h after injection of cobaltous chloride in percentage of initial value.
x——x controls ♂; o——o $CoCl_2$ 45 mg/kg.

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[6] J. H. FODDEN, Arch. Path. *61*, 65 (1956).

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Table I
Blood sugar changes during 24 h after injection of cobaltous chloride in percentage of initial value

	Before injection mg/100 g	Blood sugar change in percentage of initial value (h after injection)								
		1	2	3	4	5	6	9	12	24
Controls ♂	92	+2·0	−5·8	+1·8	−1·9	−3·0	+1·5	+1·8	−0·4	−3·3
$CoCl_2$ 45 mg/kg ♂	78	+83·4	+109·5	+78·9	+42·4	−0·9	−11·9	−21·7	−17·2	−2·8
d.f.		48		47		47		46		22
t		10·86***		8·75***		1·07		3·12**–***		0·056
$CoCl_2$ 20 mg/kg ♂	92	+12·9	+5·5	−5·4	−10·9	−14·2	−21·2	−24·6	−19·7	−18·2
d.f.		48		47		47		46		22
t		1·613		1·535		3·08**–***		3·66***		1·59
Controls ♀	78	+6·5	+3·8	+1·9	+2·4	+8·6	+1·5	−11·2	−0·9	+1·6
$CoCl_2$ 45 mg/kg ♀	73	+131·5	+103·3	+72·5	+75·8	+6·2	−4·7	+16·3	−3·1	+2·0
d.f.		23		24		23		24		11
t		4·13***		2·93**–***		0·346		1·046		0·034

* = probable; ** = very probable; *** = significant; *d.f.* = degrees of freedom; *t* = Student's *t*-test.

Material and Methods.—Rats of both sexes were given cobaltous chloride subcutaneously in doses of 20, 35, 40, and 45 mg/kg of body weight and the blood sugar was determined by Hagedorn and Jensen's method once an hour for 6 h and subsequently at longer intervals for 15 days. The animals were sacrificed at different times after the cobaltous chloride injection and the pancreas was fixed in Bouin's fluid and stained by the Gomori method with chromhematoxylin–Ponceau-fuchsin or paraldehydefuchsin–Ponceau-fuchsin. Karyometric examination was carried out by tracing off the nuclear surface of α- and β-cells at a magnification of approximately 2000 and determining the area with a planimeter.

Results.—Following the subcutaneous injection of cobaltous chloride in a dose of 45 mg/kg of body weight a considerable increase in the blood sugar was recorded over a few hours (Table I, Fig.). The finding was the same for both males and females. After 35 or 40 mg/kg, the blood sugar level was also elevated whereas the 20 mg dose did not cause any statistically certain hyperglycemia. About 6–12 h after injection, a hypoglycemic effect was noted in male rats both after 45 and 20 mg/kg of $CoCl_2$ but not in the females. No disturbances were observed in the blood sugar level at a later stage after the injection (Table II).

The result of the nuclear measurements is shown in Table III; no statistically certain disturbances were demonstrated in the α-cells but there was a slight decrease of the nuclear area in the β-cells 24 h after the injection.

Discussion.—The hyperglycemia observed in this investigation shows a correspondence with the findings obtained by MUKHERJEE *et al.*[7] in rats. In addition, it was established that doses of 35 and 40 mg/kg of body weight give similar results.

Table II
Blood sugar changes in percentage of starting value over 15 days following injection of cobaltous chloride

	Before injection mg/100 g	Blood sugar change in percentage of starting value (days after injection)		
		2–4	5–8	14–15
Controls ♂ . . .	89	+13·5	+7·3	−2·4
$CoCl_2$ 45 mg/kg ♂	77	+23·2	+8·5	+3·9
d.f. . . .		55	42	20
t		1·23	0·214	0·565
$CoCl_2$ 20 mg/kg ♂	87	+7·8	−2·7	−13·0
d.f. . . .		56	43	20
t		0·81	1·87	1·044

The possibility that these blood sugar changes might be due to primary disturbances in the cells of Langerhans' islets is contradicted by the karyometric results. It may perhaps be considered remarkable that the appreciable

Table III
Karyometric investigation of α- and β-cells. Nuclear area in μ^2

	α-cells			β-cells			β/α	*d.f.*	*t*
	mean	*d.f.*	*t*	mean	*d.f.*	*t*			
Controls	25·5			28·8			1·13		
$1^1/_2$ h after $CoCl_2$ inject.	25·1	8	0·552	28·7	8	0·182	1·14	8	0·303
3 h after $CoCl_2$ injection	25·6	7	0·106	28·1	7	1·051	1·10	7	0·772
6 h after $CoCl_2$ injection	25·8	7	0·318	28·7	7	0·164	1·12	7	0·21
12 h after $CoCl_2$ injection	25·0	8	0·521	26·9	8	1·96	1·08	8	1·296
24 h after $CoCl_2$ injection	25·1	8	0·525	27·2	8	3·34**	1·09	8	1·093

blood sugar disturbances did not produce any effect on the α-cells and caused only a slight reaction in the β-cells, from what can be judged from the karyometric results.

There would thus seem to be some reason for assuming that the hyperglycemia has some extra-insular source. Disappearance of the blood sugar elevation has also been observed after cobaltous chloride administration in experimental animals with excised adrenal glands[8,9], or after preceding treatment with dihydroergotamine[10], and it therefore seems probable that in rats also the effect produced by cobaltous chloride might be in some way connected with the adrenal glands.

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Résumé

L'effet de l'administration de chlorure de cobalt chez le rat normal est une hyperglycémie prononcée dans l'espace de quelques heures après l'injection. Il n'est pas possible de déceler des changements dans les cellules α des îlots de Langerhans. Les noyaux des cellules β ne montrent qu'une diminution faible après 24 h de l'injection.

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Dehydrogenase Activity of *Borrelia recurrentis*

So far, little is known about the metabolism of pathogenic spirochetes, since their mass cultivation *in vitro* in a defined medium has only been partially successful[1,2]. Recently, BUCCA *et al.*[3] and BARBAN[4] reported about certain metabolic observations; however, they used in their studies the non-pathogenic Reiter strain of Treponema for testing.

In the present work, the dehydrogenase activity of a known pathogen, *Borrelia recurrentis*, was investigated in a fairly well defined medium, using Tetrazolium reduction as an indicator.

Suspensions of washed *Borrelia recurrentis*[5], containing 10^5–10^7 spirochetes per mm^3, were prepared as follows: Mice infected with the organisms were exsanguinated and the blood placed in an anticoagulant fluid (1 p sodium citrate (3%), 1 p saline (0·85%) and 2 p tryptose-phosphate broth (Difco)). The spirochetes were sedimented by spinning and resuspended in fresh fluid (2 p saline and 2 p tryptose-phosphate broth). Several washings were then made, to remove all extraneous materials. The washed spirochetes remained viable for at least 12–15 h in the wash fluid without added nutrients.

The various dehydrogenase systems to be tested were made up in test tubes containing (1) 0·5 ml of the spirochete suspension; (2) 0·1 ml of a 1% solution of one of a number of substrates (glucose, pyruvate, succinate, fumarate, lactate, formate, glutamate, and asparagine); (3) 0·1 ml of a 0·05% solution of either cysteine, cystine, oxidized or reduced glutathione, or ascorbate; (4) 1 ml of freshly prepared 1:10 dilution made from a 1% stock solution of 2, 3, 5 triphenyltetrazolium chloride in phosphate-saline buffer, pH 6·85 (TTC); and (5) saline (0·85%) to bring the total volume up to 2 ml. In the controls, either the borrelia suspension or one of the substrate components was replaced by buffered saline. All tubes were layered with petrolatum and incubated in a water bath for periods up to 6 h at 30°C. Higher incubation temperatures tended to have detrimental effects on the spirochetes. It was also observed that a pH of 6·85 eliminated the troublesome auto-reduction of the TTC, which frequently occurred at a pH of 7, or above.

Results.—On preliminary examination, the dehydrogenase systems concerned with oxidation of all substrates were found either equivocal or negative. After ruling out the possibility that inadequacy of pH was responsible for these results, a deficiency of appropriate coenzymes was considered. It has already been noted that addition of fresh serum somewhat enhanced activity of the borrelias. However, addition of DPN (0·1%) only slightly improved the results. Apparently, during preparation of the microbial suspension, sufficient coenzymes were carried over so that additional DPN had but little influence.

Since it is known that SH-compounds have the ability to activate several dehydrogenase systems, compounds such as cysteine, cystine, reduced and oxidized glutathione were added to the various test systems. The reaction which was previously weak and unduly protracted (requiring overnight incubation) became accelerated in presence of the reduced agents (less than 6 h) but remained inactive with the oxidized form of compounds. Under the experimental conditions employed, the most active enzyme was one attacking the formate; but following it, the other substrates were also oxidized after variable lapse of time.

In the controls, in the presence of heat-killed organisms, no reduction occurred. Similarly, omission of either of the substrates or of the borrelias failed to reduce the dye.

It remained to be seen whether the SH-compounds acted specifically or not. Using ascorbate as an alternate reducing agent, the very same results were obtained as with SH-compounds. Hence there can be little doubt that the catalytic effect is not specific and is not limited to SH-compounds alone.

The activation phenomenon may be explained by assuming that the enzymes became inactivated in the course of preparation (due to oxidation or perhaps due to trace metal poisoning), and reactivated by adequate reduction or neutralization of the metal poisons.

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Zusammenfassung

Es wurde gezeigt, dass *Bor. recurrentis* unter üblichen Bedingungen nur eine schwache Fähigkeit besitzt, verschiedene Substrate zu dehydrogenieren. In Gegenwart von reduzierenden Agentien (wie SH-Verbindungen oder Ascorbinsäure) wird diese Aktivität hingegen bedeutend verstärkt.

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