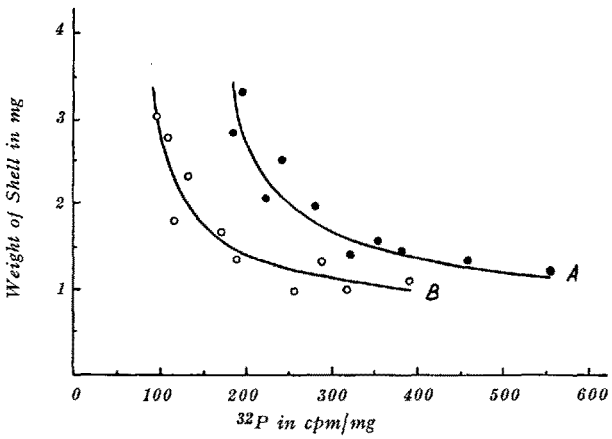
exchange of phosphorus in the shells of these mollusca could be determined. It was mainly the P-metabolism of growing animals which was studied in our experiments. Thus we found, e.g. with the type *Physa acuta*, that approximately 50% of the accepted ^{32}P-isotope changes in its shell in the course of 5 days. A rather peculiar observation was made that the percentage of the ^{32}P exchanged in the shells was the same, even though individuals of different shell-weight, and thus of different age, were used. At the same time the absolute quantity of the bound ^{32}P was indirectly proportional to the shell-weight, i.e. the ligther the shell the more ^{32}P was bound by it. This phenomenon can easily be explained by the more intensive metabolic activity of young growing animals. The following Graph shows the uptake of ^{32}P in 5 days (*A*), and at the same time its exchange during the following 5 days (*B*), in shells of the *Physa acuta*.



cpm/mg = counts per min per mg weight of snail-shell.
Controls performed with shells containing no animal did not show any significant uptake of radiophosphorus during 5 days.

Methods.— 20 young mollusca of different size were grown for 5 days in a medium (100 ml of tap-water from which Cl_2 were eliminated) containing 1 μc/ml ^{32}P (obtained by neutralization of H_3PO_4 by NaOH). After 5 days all the animals were taken out of the medium and washed briefly with distilled water 10 times until the distilled water showed no more radioactivity. 10 of them were killed in boiling distilled water, the bodies were shelled and the shells, after washing 3 times with distilled water, dried at 60° for 24 h (the part of shells designated with *A* in the Graph). The other 10 animals, after 1 h in pure tap-water, were transferred to the same jar under the conditions described above, but without any ^{32}P. After 5 days, these 10 snails were killed and dried in the same manner as is described above (the *B* group in the Graph). Then the shells of both groups were weighed and ground to powder, and the activity of 1 mg of the shell-powder was estimated with a G.M. counter.

Because it is well known that phosphorus does not belong to the basal building material of the mollusca-shell, our experiments seem to confirm the old conception of phosphorus being the agent which transfers calcium to the shells[1]. This conception supposes in principle that calcium is transferred to the organic layers of the shell, perhaps as soluble calcium diphosphate, and there this crystallized compound is readily changed into calcium carbonate, the real building matter of the shell. Phosphate is then brought back to the normal snail metabolism. If this conception were right, then the whole calcium transfer by means of phosphorus would be far more complicated than is usually supposed in the ossification of mammals[2].

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Zusammenfassung

In Schalen von wachsenden Wasserschnecken, *Physa acuta*, wurde binnen 5 Tagen 50 % Austausch des Radiophosphors ^{32}P beobachtet. Auch wurde festgestellt, dass das absolute Quantum des ^{32}P, der binnen 5 Tagen aufgenommen wird, indirekt proportional dem Schalengewicht ist, das heisst, je schwerer die Schale ist, desto mehr ^{32}P wird aufgenommen.

[1] H. SIMROTH and M. HOFFMANN, in: *Dr. H. G. Bronn's Klassen und Ordnungen des Tierreiches*, III. Bd. (Mollusca), II. Abt., 2. Buch (1928), p. 192.

[2] TH. BERSIN, *Kurzes Lehrbuch der Enzymologie*, 4. Aufl. (Akad. Verlagsges., Leipzig 1954), p. 297.

Streptomycin and Endocrine System

In a previous work[1] we published data that streptomycin and inositol (which forms a part of the streptomycin molecule), injected into healthy rabbits produces a rise in the neutral blood fat content; and the same is seen, though in a lesser degree, in persons treated with the therapeutic dose of streptomycin.

We believed that it is mainly the function of one or several members of the hypophyseal-hypothalamic-adrenal system which should be considered as probable cause of the changes in the fat metabolism which we established. This assumption can be sustained by several clinical observations described in the complications of streptomycin treatment as the manifestation of hypertrichosis (FONÓ[2]), the appearance of striae atrophicae (BOQUIEN and others[3]), hyperglycaemia, acne, retention of sodium, the decrease of the number of circulating eosinophils and lymphocytes (BARNARD[4]), signs which may also develop as clinical features of different endocrine disorders.

Recently KÁROLYHÁZI[5] in our clinic found that the diabetic state in alloxan induced rats was significantly deteriorated by the administration of streptomycin.

To elucidate the mechanism of this action of streptomycin, systematic researches were begun with the investigation of the possible changes in the function of the anterior pituitary.

The first experiments were performed on 12 healthy rabbits kept on the same diet, and the changes of the dextrose, dextrose + insulin, dextrose + insulin + vitamin B_1 and finally dextrose + vitamin B_1 tolerance tests were determined before and after treatment. The resul-

[1] L. MOSONYI *et al.*, Lancet 2, 81 (1951); Wien. Z. inn. Med. *33*, 384 (1952).
[2] R. FONÓ, Orv. Lapja *5*, 515 (1949).
[3] Y. D. BOQUIEN *et al.*, Bull. Soc. méd. Hôp. Paris *1948*, 852.
[4] R. BARNARD, Lancet *1*, 612 (1952).
[5] GY. KÁROLYHÁZI, Orv. Hetil. *94*, 34 (1953).

Rabbit No.	Steroid excretion (*A* 17-ketosteroid, *B* 11-oxysteroid) mg in 24 h							
	before				after			
	Streptomycin treatment							
			Response to ACTH				Response to ACTH	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	0·7	0·14			1·15	0·64	0·95	0·70
2	0·55	0·35			0·61	0·36	0·99	0·76
3	0·61	0·37			0·61	0·45	0·69	0·45
5	0·65	0·085			0·16	0·13	0·3	0·30
6	0·9	0·21	2·0	1·23	2·4	0·26	1·8	0·36
7	1·35	0·38	2·0	0·75	1·15	0·1	0·95	0·15
8	–	0·45	2·0	1·1	2·0	0·18	1·2	0·23
9	1·7	0·4	2·3	1·25	0·76	0·08	0·9	0·18
10	4·1	0·66	4·1	0·89	1·05	0·15	2·15	0·17
11	–	0·5	–	0·9	1·9	0·29	0·5	0·22
Mean (Rabbit No. 10 omitted)	1·05 ± 0·13	0·37 ± 0·06 Sign. Diff. = 6·6	2·1	1·03 ± 0·08 Sign. Diff. = 6·6		0·28 ± 0·05		0·37 ± 0·08

ting curves were evaluated through planimetric comparison. The experiments were carried out using streptomycin and inositol separately in the two series.

Between the two series of tests, for a period of 3 weeks we daily injected intramuscularly 15 mg streptomycin or 6·7 mg inositol (equimolecular with 15 mg streptomycin), per kg body weight to both groups of rabbits. There were no complications at all and the animals tolerated the injections well. Similarly to our earlier findings, a significant rise in the neutral blood fat content was found in the majority of the rabbits.

It could be established from the 4 series of experiments that there is no definite change in the secretion of the carbohydrate regulating factors of the anterior pituitary during streptomycin treatment. The same evidence could be concluded concerning the function of the STH and ACTH. 40 young male albino rats were injected throughout 6 weeks with 15 mg streptomycin per kg body weight daily. Neither in the length of the tails, nor in the body weight, nor in that of the liver and adrenals could any significant difference be detected between streptomycin-treated and control animals. It would be illogical, therefore, to maintain that the anterior pituitary plays the chief role in the "general effect" of streptomycin.

Recently male rabbits were injected in the same manner with streptomycin and determinations of the blood sugar, potassium, sodium, neutral fat and cholesterol content of the serum were performed simultaneously with measurements of the 24 h 17-ketosteroid and 11-oxysteroid excretion before and after the injections. It could be concluded that there is a significant change in the adrenal cortex activity following streptomycin treatment. The body weight rose in 8 of the 11 rabbits, the serum potassium content fell, the serum sodium level rose in all cases and so did the blood sugar, the neutral fat and also the cholesterol content of the serum in the majority of the animals. The greatest and most uniform change could be observed in the response to ACTH administration (Table).

As CLAYTON and HAMMANT[6] stated, guinea pigs respond to ACTH regularly with 1 mg elevation of the 24 h excretion.

The 17-ketosteroid excretion usually responds with lesser increase on the administration of ACTH, than that of the 11-oxysteroid, though the effect of ACTH on cholesterol favours the production of pregnenolone and the conditions to produce 17-ketosteroids (RENOLD[7]).

The excretions prior to the antibiotic treatment differ principally from those after the administration of the antibiotic.

(a) Before the treatment the response to ACTH is normal in the excretion of 17-ketosteroids as well as in that of the 11-oxysteroids.

(b) After the prolonged treatment the response in the excretion of 17-ketosteroids shows an increase in 5 cases, in 5 a fall.

(c) After the streptomycin treatment the rise in the excretion of 11-oxysteroid in response to ACTH is lessened.

The excretion of cortical steroids follows an intra-adrenal enzymatic activity (BISHOP[8]), the impairment of which may result in an unusual relation between the two groups of hormones. In another series of experiments on *in vivo* perfusated dog adrenals[9], we found, indeed, that the acutely administered streptomycin has an impairing action on these processes. This often results in a lessened secretion of both hormones, in other cases there is a reciprocal relation between the two hormones: the production of one hormone is raised, of the other diminished.

From the above experiments, it can be concluded that the prolonged administration of the antibiotic may result in a similar impairment. This is manifested in the following directions: (1) the increase in electrolyte retaining capacity, (2) the irregular response to ACTH in the 17-ketosteroid excretion, (3) the lessened degree of response in the 11-oxysteroid production.

These results cannot be invalidated by the fact that there is no constancy in the 11-oxysteroid excretion of different individuals, because there is no reason to infer the same changes in the ACTH responsiveness.

The two types of responses explain the variations in the clinical signs referred to. It is not necessary that any clinical manifestation whatever be present. However, if the excretion of androgenic hormones prevail, hirsutism may appear; if the antibiotic interferes with the produc-

[6] B. E. CLAYTON and I. E. HAMMANT, Nature *176*, 401 (1955).

[7] A. E. RENOLD *et al.*, J. clin. Endocrin. *12*, 763 (1952).

[8] PM. F. BISHOP, *Recent Advances in Endocrinology* (Cameron) (J. A. Churchill Ltd., London 1954).

[9] L. MOSONYI, M. MINCSEV, L. POLLÁK, and E. NÉMETH (in press).

tion of androgenes, the resulting higher (or relatively higher) glucocorticoid production may give rise to Cushingoid signs.

Observations in human beings are in concordance with the above data.

A 30 years old woman was treated abroad against her febrile illness with streptomycin. The treatment lasted several weeks and the patient got approximately 30 g of the antibiotic. Following the cure hypertrichosis developed. No endocrinological abnormality could be detected for this phenomenon, save the elevated 17-ketosteroid excretion (13 mg). The 11-oxysteroid excretion amounted to 0·76 mg. The mean, normal value in 17-ketosteroid excretion of our female patients is 10·85 mg. According to this the value lies in the upper range. In contrast to which, the 11-oxysteroid excretion was unusually low.

Finally the impairment of the enzymatic processes of the adrenal cortex may result, as DEBRÉ[10] states, in the hyperactivity of the anterior pituitary. This may manifest itself in deterioration of the alloxan-induced diabetes in rats, as experienced by KÁROLYHÁZI.

The clinical and the experimental evidence leads to the conception that interference with the adrenocortical enzyme function is responsible for the endocrine signs in streptomycin therapy.

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Zusammenfassung

Auf Grund klinischer Erfahrungen, nach welchen anlässlich einer Streptomycintherapie verschiedene endokrine Störungen beobachtet werden konnten, wurden zur Klärung derselben Tierexperimente durchgeführt. Die Versuchsergebnisse deuten auf eine funktionelle Störung der Nebennierenrindenenzyme hin, hervorgerufen durch die Einwirkung des Antibiotikums, wobei die verschiedenen klinischen Symptome als Manifestationen der Verschiebung der normalen Hormonverhältnisse aufgefasst werden.

[10] R. DEBRÉ *et al.*, Pr. méd. *85*, 583 (1954).

Diagnostic Test for Cancer

During an investigation of possible chemical changes in the spleen of rats following weekly injections of chemical carcinogens over a seven weeks period, it was noted that the lipid fraction of the spleen of injected rats had a distinctly darker yellow color than did similar fractions of normal rat spleen. The lipid fraction from a transplanted rat sarcoma had the same color as that of the spleen following treatment of rats with 1, 2, 5, 2-dibenzanthracene (DBA).

This observation leaving to attempts of characterization of the phospholipids from different tumors and from spleen after treatment of rats with various carcinogens and non-carcinogens, was described by HAKIM[1].

In addition to immunological studies by chromatographic and paper ionophoretic techniques, a modification of the Feulgen reaction was found to be useful in the identification and characterization of the new phospholipids induced by chemical carcinogens in the spleen. These new phospholipids could also be revealed at the same time in serum.

This new abnormal acetat-lipid, could be tested for in the serum of tumor carrying animals, and recently it was revealed in all *human cancer cases* tested by 2 techniques, in the following way:

Modified Feulgen Reaction.— To 500 µl of the serum the following reagents are added in the order given:

10 µl of 0·5 % Ascorbic acid,
20 µl of 0·5 % Thiamine HCl,
100 µl of 95 % alcohol,
150 µl of 10 % KOH
100 µl of 0·5 % $CuSO_4$
120 µl of 0·02% fuchsin (basic fuchsin).

The mixture is shaken after each addition. The color is noted between 15–30 min in the sun light.

Normal sera gave a *red* color, while sera from tumor bearing animals, and from *human cancer cases* when tested in a similar manner gave a *purple* color.

Paper Electrophoresis.— Electrophoresis was accomplished in the LKB electrophoretic equipment, using 40 × 410 mm Schleicher and Schuell No. 2043 B (120 g/m²) filter paper, or Whatman No. 1 filter paper. Veronal buffer, pH 8·6 with ionic strength 0·125, or Veronal-borate buffer (equal volumes of veronal buffer pH 8·6, ionic strength 0·060 ionic strength, and Borate buffer pH 8·6, 0·1 *M*). The test is carried at room temperature (about 18°C), with a potential of 200 V across the filter paper, and a current of 8 mA, for 24 h. After electrophoresis, the electrogram was dried and stained for proteins with bromphenol.

A more general survey is in progress, to determine the possible physiological conditions, on either secondary infections, or complications with cancer which might alter the above described reactions. Similar studies are in progress to determine whether fibrinogenic, or infectious disease might give a similar reaction, in non-cancer, non-normal persons.

The presence of an abnormal gamma globulin in the sera of human cancer sera is evident. Typical electrophoretic tests, presented in the joined Figure include

[1] A. A. HAKIM, Exp. Med. Surg. (in press); Proc. Amer. Ass. Cancer Res. *1956*, April 15.

Serological Reactions for Cancer

	Modified Feulgen reaction			Paper ionophoretic reaction		
	Total Number Tested	Positives	Negatives	Total Number Tested	Positives	Negatives
normal persons	100	2	98	100	100	100
known Cancer persons	75	75	0	75	75	0