anti-thyroid drugs (thiourea) at a 0·5% level in the diet. The drug was fed first at a level of 0·01% and gradually increased to the 0·5% level. This latter procedure prevented the acute pulmonary oedema known to occur especially after feeding thiourea. The diet was otherwise the same for these experimental rats as for the control series. The remaining 101 rats, constituting the control series, were treated with trypan blue only and were not fed any of the antithyroid drugs. Since 3 of the rats fed thiourea and 5 of the control rats died before the 100th day of the experiment, only 17 of the experimental rats and 96 controls could be reported as the effective number to be used for comparison.

71 of 96 control rats, that is 76% developed reticulosarcoma whereas not a single experimental rat developed such a tumour. These results are statistically significant.

Examination of the livers of the trypan blue-treated rats fed antithyroid drugs revealed that whereas tumour formation was inhibited, only in one instance was the trypan blue reaction completely suppressed. In the remaining 16 livers, there was mild portal reticulosis, and the appearance of fluid-filled cysts, similar to those described previously[1]. However, with one exception, the trypan blue response in the rats fed antithyroid drugs at the end of 200 to 400 days was not greater than that observed in control animals at the end of the 40th day of the experiment. In the exceptional instance, the reaction at the 140th day was comparable with that occurring in the control rats at about the 70th day of treatment.

From the foregoing, it can be concluded that a measure of thyroid activity is essential for the development of reticulo-sarcoma in response to trypan blue. Depression of thyroid function below a defined level, whether achieved by antithyroid drugs or by underfeeding, will not only depress the intensity of the reticulosis but indeed excludes the development of reticulo-sarcoma. Depression of the thyroid therefore retards and can even prevent the emergence of experimentally induced reticulo-sarcoma as well as carcinoma of the liver and of adenoma of the pituitary gland.

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Zusammenfassung

Die Entstehung von Reticulosarkom bei Ratten nach mehrmaliger Injektion von Trypanblau kann unterdrückt werden durch Entkräftung, Verabreichung antithyreoidaler Mittel und durch alle andern Faktoren, welche den Stoffwechsel herabsetzen.

[1] J. GILLMAN, T. GILLMAN, and C. GILBERT, S. Afr. J. Med. Sci. *14*, 21 (1949).

The „Feeding Centre" of the Hypothalamic Region of the Rat Brain

According to BROBECK *et al.*[1], the sense of hunger and the food intake is regulated through a "feeding centre" localized in the lateral parts of the anterior hypothalamus. An attempt to analyse the biochemical mechanism of the regulation was made, applying the tracer technique. Groups of hungry (H) rats were administered 200 μC $Na_2HP^{32}O_4$ intraperitoneally at the end of a 24 h hunger-period, together with fed (F) ones. They were sacrificed 15–60 min later through plunging in liquid oxygen. In other sets of experiments the isotope was given at the start of the hunger period and the animals were sacrificed at the end. Besides the feeding centre, C, two adjacent parts of the hypothalamus, denoted A and B, were prepared from the frozen brain tissue, likewise samples of cerebrum, blood, liver and muscles were taken for comparison of the gross distribution of activity in H and F animals.

Pooled brain samples of H and F rats were analyzed with regard to:

(a) the distribution of P^{32} per unit volume of the samples;
(b) the partition of P^{32} on the trichloroacetic acid (TCA) fraction, the lipid fraction and the combined protein + nucleic acid fraction;
(c) the partition of P^{32} within the TCA-fraction[1].

Determinations of total phosphorus in the A–C samples and of orthophosphate and the hydrolysable end-groups of ATP+creatine phosphate, as well as of total creatine[2] (free creatine+creatine phosphate) in the TCA fractions, were also performed.

The liver of H rats took up appreciably more ^{32}P than the liver of F animals, whereas the concentration in the muscles, the cerebrum and the blood was similar within experimental errors. This suggests that the distribution of ^{32}P through the blood to other parts of the brain than the hypothalamus does not differ in the two groups of animals (Table I).

The distribution of P^{32} on the hypothalamic samples under consideration shows a characteristic pattern (Table I) which may be described in the following way: while the *C* samples of H rats accumulate more activity than those of F rats, the opposite is true for samples *A* and *B*. This is simply demonstrated through the ratio's *C/A* and *C/B* which measures are statistically different in H and F rats. No significant differences occured in the amount of total phosphorus which account for these changes. The same general mode of distribution was found when the samples were chemically fractionated according to (b) and the activity of the single fractions determined. The analyses thus suggest an all-over change in the biochemical activity of the samples of hungry rats as compared with fed ones.

It is of interest to note that the integrated activity in the three samples $A + B + C$, making up about $^2/_3$ of the total hypothalamus, does not differ appreciably in the two groups of rats. This indicates that the P^{32} supply through the blood to the hypothalamic region as a whole is rather unimpaired by the state of hunger. Obviously, then, the partition of activity within the hypothalamus is autonomously regulated.

In further analyses, the concentration of the hydrolysable end-groups of ATP+creatine phosphate was found mainly to follow the same pattern of distribution as the P^{32} given in Table I, while the concentration of orthophosphate seems to vary less (Table II). Likewise the distribution of total creatine agreed with the general scheme. Calculations of the amount of activity present

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[1] L. ERNSTER, R. ZETTERSTRÖM, and O. LINDBERG, Acta Chem. Scand. *4*, 942 (1950).

[2] P. EGGLETON, S. R. ELSDEN, and N. GOUGH, Bioch. J. *37*, 526 (1943). – A. H. ENNOR and H. ROSENBERG, Bioch. J. *51*, 606 (1952).

Table I. – Resorption of P^{32} in the unfractioned samples *A*, *B* and *C* and incorporation of the isotope into their TCA, lipid and protein + nucleic acid (NA) fractions. Determination at 60 min and 24 h after injection of isotope. Activity measurements calculated as counts/min per mm^3 of brain sample.

Fraction analyzed	Isotope time	Hungry Counts/min/mm^3 of			Ratio of:		Fed Counts/min/mm^3 of			Ratio of:		Significance test for the different hungry: fed			
												C/A		*C/B*	
		A	*B*	*C*	*C/A*	*C/B*	*A*	*B*	*C*	*C/A*	*C/B*	*t*	*P*	*t*	*P*
Unfractioned	60 min	52·2	30·7	75·6	1·67	2·89	72·0	39·5	40·0	0·763	1·14	2·81	~ 0·01	3·88	< 0·001
sample	24 h	126	106	187	1·49	1·77	132	108	132	0·986	1·20	3·60	~ 0·001	3·79	~ 0·001
TCA		55·3	36·0	68·3	1·25	1·95	50·5	44·2	40·6	0·948	1·02	1·68	< 0·2	4·12	~ 0·005
Lipids	60 min	1·33	0·620	1·71	1·54	3·90	1·31	1·53	1·33	1·17	1·03	0·765	0·4–0·5	2·96	< 0·02
Proteins-NA's		4·69	3·22	6·09	1·32	2·07	3·64	3·77	3·63	1·19	1·10	0·608	0·5–0·6	2·33	< 0·05
TCA		81·4	62·5	76·7	0·954	1·23	87·0	63·4	50·0	0·558	0·790				
Lipids	24 h	20·9	18·2	28·4	1·28	1·61	24·5	18·9	21·2	0·884	1·12				
Proteins-NA's		14·5	12·8	17·5	1·22	1·38	14·7	12·8	13·2	0·903	0·947				

in the fractions also agreed in principle with the results given in Table I. Attempts to analyse creatine phosphate gave inconsistent results because of the small amounts present.

In particular the mode of partition of ATP and creatine between the various samples is interesting in view of the importance of these substances in the energy metabolism of the cell, NACHMANSOHN and MACHADO[1] have shown that homogenized brain tissue synthezises acetylcholine (Ach) in the presence of ATP. An increase of Ach would be expected to cause local vasodilatation and consequently also an increase in the ^{32}P supply. Considering these findings, one would expect the variations found in the ATP and creatine concentration to regulate the supply of P^{32} to various parts of the hypothalamus. In experiments not recorded here, about 90% of the activity was still found to be present as ortho-P^{32} 30 min after the injection. The partition of ortho-P^{32} between the various samples thus agreed with the corresponding determinations given in Table II, while the variations in the total amount of orthophosphate were less pronounced. The uneven distribution of ortho-P^{32} will thus in turn account for the results found in the lipid and protein + nucleic acid fractions (Table II).

Although many details are not yet settled, it may be that the sense of hunger and the food intake is regulated through a mechanism where the reactions proposed by NACHMANSOHN play a predominant role. The analyses also indicate that biochemical differences do occur between closely adjacent volumes of brain tissue. This is in line with the notion of HIMWICH[1] that specialized parts of the brain are distinguished by a chemical activity of their own.—A full account of these biochemical investi-

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Table II. – Concentration of ortho-P and hydrolyzable end-groups of ATP and creatine phosphate (ATP-P + creatine-P) expressed as γ P $\times 10^{-2}$ per mm^3 of tissue. Total creatine (free creatine+creatine phosphate) as substance per mm^3. P^{32}-activity calculated on the total amount of each fraction present per mm^3 of tissue. Mean values from determinations on 12 rats 24 h after the start of the hunger period and injection of P^{32}.

State of animals	Samples analyzed	Fraction from TCA-extract	Concentration determinations				Activity determinations			
			γ	$\Sigma A+B+C$	*C/A*	*C/B*	counts/min	$\Sigma A+B+C$	*C/A*	*C/B*
Hungry	A	Ortho-P	52·7	151·5	0·93	0·97	25·1	73·1	1·08	1·32
	B		50·4				20·7			
	C		48·4				27·3			
Fed	A		63·7	166·8	0·74	0·85	32·1	80·5	0·66	0·79
	B		56·4				27·1			
	C		46·7				21·3			
Hungry	A	ATP-P+ creatine-P	11·4	30·5	1·24	3·36	0·451	1·35	1·30	1·93
	B		5·03				0·306			
	C		14·1				0·588			
Fed	A		5·84	28·8	1·42	0·56	0·392	2·27	0·97	0·25
	B		14·7				1·50			
	C		8·21				0·379			
Hungry	A	total creatine	1·45	4·96	1·31	1·19				
	B		1·60							
	C		1·91							
Fed	A		1·52	4·28	0·85	0·88				
	B		1·47							
	C		1·29							

gations together with physiological ones has appeared elsewhere[1].

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Zusammenfassung

Die Analyse mit radioaktivem Phosphat zeigte, dass das «feeding centre» (BROBECK) im Hypothalamus von Ratten, die 24 h ohne Futter gehalten wurden, erheblich mehr P^{32} aufnahm als das gesättigter Tiere. Der Aktivitätsüberschuss war gleichmässig auf die säurelösliche, Lipoid- bzw. Protein + Nukleinsäure-Fraktion verteilt. Es wurde weiter gezeigt, dass ATP und Kreatin eine Konzentrationsverschiebung wie P^{32} aufweisen. Es wird angenommen, dass die ungleichförmige Verteilung von P^{32} eine Folge der ATP-Kreatin-Verschiebung im Hypothalamus ist.

[1] A. FORSSBERG and S. LARSSON, Acta Physiol. Scand. *32*, Suppl. 115, Part II (1954).

The Uptake of Radioactive Phosphorus into Phosphorus Compounds in the Brains of Virus-Infected Mice[1]

Since viruses can multiply only in living tissues, it is of great interest and importance to investigate the changes in infected tissues. The tracer-technique with radioactive isotopes has been proved to be a useful procedure for the minute detection of biological and chemical processes. Several reports have been presented on the distribution of radioactive phosphorus (P^{32}) in the brain tissue of normal or virus-infected animals[2]. The purpose of this paper is to add some findings relative to the subject, which were obtained by the authors after conducting a preliminary experiment[3].

Material and Methods

White mice weighing 6·5 ± 0·3 gm and the mouse-adapted Mochizuki-strain dengue virus[4] were used. This virus has been able to proliferate regularly in mouse brain tissue. The mice were fed on wheat and green vegetable throughout the experiments.

From a number of mice inoculated with the virus intracerebrally, 6 groups of 4 or 5 mice each were picked at 4, 28, 52, 76, 100, 124 h, respectively, following the inoculation. Viral activity of the inoculum was adjusted so that no mice survived at 168 h after the inoculation. Each group was then injected intraperitoneally with a solution of P^{32} [5]. The injected dosage per mouse was 0·02 ± 10% mc in 0·1 ml, at the time of administration.

24 h after each isotope-administration, the mice of each group were killed by cutting the throat. From each animal, blood was collected and the brain was removed. The materials, respectively, were pooled in each group. The blood was centrifuged at 3,000 r.p.m. for 15 min to separate the serum. Suitable precautions were taken to minimize the haemolysis. The brains were washed three times with chilled saline solution and wiped thoroughly with filter-papers, in order to eliminate the blood components as completely as possible.

Of the serum, the inorganic phosphorus fraction was separated by the method of DELORY[1]. The brains were weighed, homogenized with a grinder, and subjected to the fractionations of phosphorus compounds contained into the total acid-soluble phosphorus (AS), phospholipid (LP), total protein-bound phosphorus (TP), desoxyribonucleic acid phosphorus (DNA), ribonucleic acid phosphorus (RNA), and residual inorganic phosphorus (RS) fractions, by the methods of SCHMIDT and THANNHAUSER[2], as well as of DELORY[1]. A minor modification of the original method[2] was made so that the separation of acid-soluble fraction was performed not by the filtration but by the centrifugation, floating detritus, if any, being very slight.

Of each fraction thus obtained, a part was subjected to the total phosphorus determination by the method of ALLEN[3] modified so that conc. sulphuric acid was used in place of perchloric acid for the digestion of organic substances. With the remainder, the radioactivity was measured as follows: After being decomposed in conc. sulphuric acid, the material was added with potassium phosphate, magnesium mixture and ammonium hydroxide. The precipitate thus formed was put into an aluminium-plated dish, washed with methanol, and, after being dried in a calcium chloride jar, was subjected to beta-ray counting with a mica-window Geiger-Mueller tube. For the details of these techniques, as well as for some theoretical aspects, refer to references[4]

Of each fraction, then, "relative specific activity", i.e., the "specific activity" (counts per minutes per milligrams of total phosphorus) as per cent of that of the serum inorganic phosphorus fraction, was calculated.

As a control, the same analyses were conducted with the materials from mice injected intracerebrally with a normal mouse brain emulsion. As another control, the specific activity of serum inorganic phosphorus in the normal mice, which were given no intracerebral injection, was surveyed to examine the reliability of this index as the base of comparison. In addition, the virus-content of infected brains at the given time was titrated by the method of REED and MUENCH[5].

Results

The virus-content of infected brains, as well as the symptoms of infected mice, are illustrated in Figure 1.

The Phosphorus amount of each fraction is tabulated in Tables I and II, including additional results on the dry weight of total protein-bound fraction (Table II*b*).

[1] Aided by grants from the Japan Ministry of Education, and the Japanese Society for the Promotion of Science.

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