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.

[2] A. J. SAMUELS, L. L. BOYARSKY, R. W. GERARD, B. LIBET, and M. BRUST, Amer. J. Physiol. *164*, 1 (1951). – M. E. RAFELSON, jr., R. J. WINZLER, and H. E. PEARSON, J. Biol. Chem. *181*, 583 (1949). – J. A. ANDERSON, C. GEMZELL, L. GEMZELL, V. S. BOLIN, and L. T. SAMUELS, Proc. Soc. Exp. Biol. Med. *73*, 690 (1950). – Department of Bacteriology, University of Utah College of Medicine, Personal communication. – H. A. DE LUCA, R. J. ROSSITER, and K. P. STRICKLAND, Biochem. J. *55*, 193 (1953).

[3] S. HOTTA, T. FUJII, and I. TANABE, Virus *2*, 26 (1952), in Japanese.

[4] S. HOTTA, J. Infect. Dis. *90*, 1 (1952).

[5] PO_4 in weak HCl; neutralized with NaOH and sterilized in an autoclave. The original lots were obtained from the U.S. Atomic Energy Commission through the medium of the Japan Scientific and Technical Administrative Commission.

[1] G. E. DELORY, Biochem. J. *32*, 1161 (1938).

[2] G. SCHMIDT and S. J. THANNHAUSER, J. Biol. Chem. *161*, 83 (1945).

[3] R. J. L. ALLEN, Biochem. J. *34*, 858 (1940).

[4] G. HEVESY, *Radioactive Indicators* (Interscience Publishers, New York, 1948). – H. T. CLARKE *et al. The Use of Isotopes in Biology and Medicine* (Univ. of Wisconsin Press, Madison, 1949). – I. L. CHAIKOFF and D. B. ZILVERSMIT, Adv. Biol. Med. Physics *1*, 321 (1948).

[5] L. J. REED and H. MUENCH, Amer. J. Hyg. *27*, 493 (1938).

Table I –Serum inorganic phosphorus amount in mouse (mg/dl).

Samples*	Time after inoculation in hours	*V*	*C*
1	28	3·9	3·6
2	52	2·8	3·0
3	76	3·3	2·5
4	100	4·1	2·8
5	124	3·3	2·3
6	148	3·2	2·9

* The indications will be applied similarly in the following tables. *V:* Virus-infected groups. *C:* Control groups.

The specific activity of serum inorganic phosphorus in the normal mice are shown in Table III, indicating a rather good stability. The relative specific activities of virus-infected, as well as control groups are presented in Table IV.

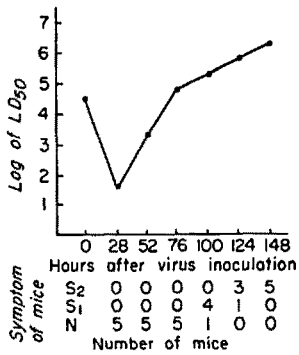

Fig. 1.–Virus-content of infected brain and symptom of infected mice. *S2*: Paralysis or severe tremor; *S1*: Debility or ruffled hair; *N*: Normal appearance.

From the figure and tables given above, it is indicated that: No significant differences were found in the total phosphorus amount of each fraction as specified here, as well as the dry weight of total protein-bound substances, between the virus-infected and control groups. During the stage of maximum virus multiplication, however, the uptake of radioactive phosphorus into phosphorus compounds increased significantly in the infected groups.

Discussion.–It has been reported that infections of neurotropic viruses stimulate the uptake of radioactive phosphorus into phosphorus compounds in the infected brain tissue[1]. The authors' studies with the mouse-adapted dengue virus also indicated that the radioactivity of the phosphorus compounds in brain tissue increased significantly during the stage of maximum virus multiplication, while no change could be detected in the total phosphorus amount. Although no conclusive evidence has yet been obtained by the present authors as to how the increase of isotopic uptake is brought about, the results may be correlated with a theory of HYDÉN[2], who, based on the ultra-violet microscopic experiments, reported that the nucleic acids are intimately involved in the process of viral multiplication.

Table II
(*b*) Dry weight of total protein-bound fraction in mouse brain (Percentages of original tissue).

Samples	*V*	*C*
1	10·6	10·5
2	10·9	10·5
3	10·2	10·2
4	11·2	12·3
5	11·5	11·7
6	10·6	10·8

Table III
Specific activity of serum inorganic phosphorus fraction in normal mice.

Samples*	Specific Activities**
A	100
B	152
C	87
D	99

* Each sample is a pool from 4 to 5 mice. ** Assuming that Sample-A is 100.

[1] M. E. RAFELSON, Jr., R. J. WINZLER, and H. E. PEARSON, J. Biol. Chem. *181*, 583 (1949). – J. A. ANDERSON, C. GEMZELL, L. GEMZELL, V. S. BOLIN, and L. T. SAMUELS, Proc. Soc. Exp. Biol. Med. *73*, 690 (1950). – Department of Bacteriology, University of Utah College of Medicine, Personal communication.

[2] H. HYDÉN, Cold Spring Harbor Symposia *12*, 104 (1947).

Table II.—(*a*) Phosphorus amount of mouse brain (mg per 100 g fresh tissue)

Samples	AS*		LP		TP		DNA		RNA		RS	
	V	*C*	*V*	*C*	*V*	*C*	*V*	*C*	*V*	*C*	*V*	*C*
1	82	91	173	179	79	76	20	20	26	27	6	8
2	104	79	178	193	64	70	16	17	23	19	10	7
3	83	100	166	187	79	80	19	19	22	20	10	8
4	87	85	190	182	71	75	20	21	21	23	6	9
5	93	90	181	193	73	84	18	22	19	25	9	7
6	89	92	172	168	69	77	16	23	20	24	5	8

* Abbreviations should be referred to the text.

Table IV —Relative specific activity of phosphorus compounds in mouse brain.

Samples	AS		LP		TP		DNA		RNA		RS	
	V	*C*	*V*	*C*	*V*	*C*	*V*	*C*	*V*	*C*	*V*	*C*
1	71·7	75·7	8·4	11·9	10·0	13·9	2·2	1·8	17·1	18·9	13·4	14·8
2	65·2	74·2	11·5	10·5	12·8	13·6	1·4	2·4	18·6	15·4	14·5	16·5
3	57·1	45·3	12·3	8·3	9·9	11·9	2·5	1·9	16·3	14·8	11·2	13·9
4	60·6	54·4	10·6	9·8	9·0	9·2	3·2	1·7	14·6	19·4	9·7	9·8
5	175·1	47·5	19·2	10·2	21·4	9·5	4·3	2·0	44·4	12·9	28·4	11·0
6	140·0	65·8	20·5	9·1	20·7	12·4	4·4	2·5	38·5	17·5	25·4	13·7

The authors wish to express their gratitude to Dr. TATSUO KARIYONE, and Dr. TOMOJI SUZUKI, of Kyoto University, for valuable advice and suggestions. Their thanks are also due to scientists of the Department of Bacteriology, the University of Utah College of Medicine, U.S.A., for critical discussions.

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Zusammenfassung

Die Verteilung von radioaktivem Phosphor im Gehirn virusinfizierter weisser Mäuse ist geprüft worden. Im Zeitpunkt maximaler Virusvermehrung nimmt die Aufnahme des radioaktiven Phosphors in den Phosphorverbindungen der infizierten Gehirne deutlich zu. Virologische und biochemische Aspekte wurden diskutiert.

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DISPUTANDUM

Possible Biosynthesis of D-lysergic Acid Diethylamide-like Compounds from Mescaline[1]

There are some data in the literature suggesting that the intact mescaline molecule is not involved directly in the model psychosis elicited by its administration.

Any one of 500 mg mescaline, 1 mg D-lysergic acid ethylamid (LAE) or 100 γ D-lysergic acid diethylamide (LSD) causes experimental (model) psychosis of similar intensity and duration in healthy volunteers. This decreasing order of dosage was related recently to the increasing affinity for wool protein of the same compounds (0, 1·1, 2·6 $\times$ 10^{-2} mM per gram wool) indicating that high activity as well as high affinity for wool are concomitant characteristics[2]. Since mescaline displays no affinity for wool, this might suggest that the intact molecule as such is not the active compound.

SCHUELER[3] as well as BLOCK, BLOCK and PATZIG[4], emphasize that the inhibition of rat brain respiration by 0·12% mescaline (substrate, glucose) as reported by QUASTEL and WHEATLEY[5] in their WARBURG-experiments is brought about after a 2–3 h (!) contact of the brain-homogenate with mescaline. Such a time lag points to the possible transformation of mescaline to an active compound[1].

BLOCK *et al.*[2] have shown that the metabolism of mescaline by the white mouse resembles that by man. Still, the peak of hallucinations in humans upon administration of mescaline and the presence of the highest amounts[3] of radioactive labelled C^{14}-mescaline in the *brain* of the white mouse do *not* coincide in time. This discordance might suggest the possibility of a transformation of the mescaline into the active compound.

Enzymatic incorporation of only 20–60 γ of C^{14}-labelled mescaline (i.e. around 1% of the amount administered) into the *liver* protein[4] of the white mouse as well as the peak of the phenomena of a model psychosis in humans on administration of mescaline coincide in time[5]; an observation not inconsistent with our hypothesis.

According to RICHTER[6], 58% of the mescaline administered to human volunteers can be recovered unchanged in the urine after 18 h; small amounts of trimethoxyphenylacetic acid were also found. This result is not contradictory to our hypothesis and suggests that only part of the mescaline might be transformed into an active compound.

2, 3, 4-trimethoxyphenylethylamine (iso-mescaline) an isomer of the symmetrical 3, 4, 5-compound (mescaline), does not cause model psychosis in normal volunteers[7] and was found to display no affinity for wool[8]; thus it behaved as an inactive compound like, for instance, mescaline itself[9]. It is also reported[10], that none of the

[1] Saskatchewan Committee on Schizophrenia Research. Supported by the Department of National Health and Welfare, Ottawa.

[2] R. FISCHER, Exper. *10*, 435 (1954); J. Ment. Sci., *100*, 623 (1954).

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[4] W. BLOCK, K. BLOCK, and B. PATZIG, Z. Physiol. Chem. *290*, 160 (1952).

[5] J. H. QUASTEL and A. H. WHEATLEY, Biochem. J. *27*, 1603 (1933).

[1] The 0·12% mescaline concentration used in QUASTEL's experiments is far above that which might occur in the organism [F. W. SCHUELER, J. Lab. Clin. Med. *33*, 1297 (1948); W. BLOCK, K. BLOCK, and B. PATZIG, Z. Physiol. Chem. *290*, 160 (1952)], and it can be argued that smaller amounts would need either an even longer time lag or would not cause an inhibition at all.

[2] W. BLOCK, K. BLOCK, and B. PATZIG, Z. Physiol. Chem. *290*, 230 (1952).

[3] These "highest amounts" are, however, relatively very small quantities.

[4] If the mescaline-protein complex itself might cause the model psychosis as postulated by B. PATZIG, Naturwissenschaften *40*, 13 (1953), an immunity towards mescaline, or at least an anaphylactic shock might be expected to occur after readministration of the drug. According to our own observations, this is not the case.

[5] B. PATZIG, Naturwissenschaften *40*, 13 (1953).

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[8] R. FISCHER, to be published.

[9] R. FISCHER, Exper. *10*, 435 (1954); J. Ment. Sci. *100*, 623 (1954).

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