The Table shows the results we obtained. It is interesting to observe that the transport of sodium is considerably enhanced by the addition of the amino acids to the glucose-Krebs solution. Among the tested amino acids, L-phenylalanine is presumably not metabolized by the intestinal tissue[11].

Recently it has been reported that also sugars which are not metabolized but actively transported increase the active transport of sodium across the intestinal wall[12]. Perhaps any actively transported substance increases the transport of sodium. This is also supported by the observations that under the conditions we tested, there exists a linear correlation between sodium transport and glucose transport as well as amino acid transport (Figures 1–3).

However, with a given amount of Na transported, the amount of transferred glucose is less when an amino acid is transported at the same time than when glucose is the only substance transferred. Therefore, the correlation between sodium transport and glucose transport does not seem to be strictly chemical in nature. The existing correlation may simply be due to the fact that in all cases the transport of sodium is a linear function of the free energy at the disposal of the intestinal epithelium, but the coefficient of proportionality is different according to the number of substances that are available for transport. More precisely, the percentage of the total available energy employed for the transport of sodium seems to be smaller the more the number of the transported substances increases.

The Table also shows that the absorption of glucose is smaller when L-alanine is present than when the amino acid is absent. Presumably L-alanine replaces in a more or less quantitative way the glucose as a supplier of the energetic requirements of the intestinal mucosa.

Riassunto. Proseguendo precedenti ricerche si mette in evidenza che esiste una correlazione anche tra trasporto di sodio e trasporto di vari amino acidi attraverso l'intestino tenue isolato di ratto albino. Se ne deduce che presumibilmente ogni sostanza capace di essere trasportata attivamente stimola il trasporto di sodio e che quest'ultima funzione diventa solo un indice dell'attività totale di trasporto dell'epitelio intestinale.

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[11] R. P. SPENCER and A. H. SAMIY, Amer. J. Physiol. *200*, 501 (1961).
[12] S. G. SCHULTZ and R. ZALUSKY, Biochim. biophys. Acta *71*, 503 (1963).

Examinations of the Bactericidal Properties of the Serum Against Gram-positive Microbes in Patients Suffering from Leukaemia, Malignant Lymphoma, Myeloma and Myelofibrosis

Much less attention has been paid to the study of bactericidal properties of the serum against gram-positive microbes than to the study of bactericidal properties of the serum against gram-negative microbes (system of properdin-complement). A factor responsible for the bactericidal properties of the serum against gram-positive microbes has so far been studied to some extent[1–6]. There were various synonyms used for this factor, most frequently that of β-lysin. In accordance with STERZL's[5] view, we prefer to use the term bactericidin, which was coined previously by MACKIE and FINKELSTEIN[2].

We examined the bactericidal capacitiy of the serum against gram-positive microbes in 137 patients suffering from haemoblastoses. Some of the patients were examined twice in an interval of 1 month. The group comprised 23 patients with acute leukaemia, 21 patients with chronic myeloid leukaemia, 23 with chronic lymphadenosis, 26 with Hodgkin's disease, 20 with reticulosarcoma along with lymphosarcoma, 10 with myeloma and 14 with myelofibrosis. The results were compared with a control group of 42 healthy subjects. *B. anthracoides* was used as testing microbe. A suspension of spores diluted in phosphate-saline containing a known number of spores (usually 50000) was placed in 1 ml of saline and 1 ml of serum examined was added. This mixture was stirred and incubated at 37°C. At the end of 4, 6 and 24 h 0.1 ml of cultured mixture was pipetted to 4.9 ml of physiological saline and further diluted in geometrical series. From two adjoining test tubes containing dilutions presumed, by experience, to contain countable numbers of cultured microbes, 0.5 ml were withdrawn and inoculated in 2 agar plates. The plates were dried and were incubated for 24 h at 37°C. Colony counts were then referred to the amount contained in 1 ml of serum. The mean value of both the results was taken. The bactericidin values were expressed by the index X_h/X_o, where X_h gave the number of microbes after incubation with serum at the end of the respective hour h, and X_o the original germ count entering the reaction. The more powerful the bactericidal effect of the serum, the more inhibited was the growth of the microbes, and the value of the index decreased. The highest bactericidal effect was observed in the first hours; after 24 h it was in most cases no longer notable.

Results. A statistically significant increase in the mean of bactericidin, i.e. lower values of bactericidal index, was found in almost all diagnostic groups when compared with a control group of healthy subjects. Only in chronic lymphatic leukaemia was the mean value of bactericidin not increased (Table I and II, Figure 1 and 2).

In most of the patients exhibiting increased bactericidin levels, no relation of the activity of the haemoblastic process to therapy, presence of an associated infectious process of bacterial origin, overall leukocyte counts or to abnormalities of differential leukocyte counts

[1] J. LIBÁNSKÝ and Z. JEŽKOVÁ, Neoplasma, in press.
[2] T. J. MACKIE and M. H. FINKELSTEIN, J. Hyg. *32*, 1 (1932).
[3] Q. N. MYRVIK, Ann. N.Y. Acad. Sci. *66*, 391 (1956).
[4] R. C. SKARNES and D. W. WATSON, J. exp. Med. *104*, 829 (1956).
[5] J. ŠTERZL, A. LANC, and Z. TRNKA, Cs. Mikrobiologie *3*, 348 (1958).
[6] J. ŠTERZL, J. KOSTKA, and A. LANC, Folia mikrobiol. *4*, 280 (1959).

Table I. Statistical evaluation of the values of bactericidal index *4 h* after incubation of the serum with microbes (*B. anthracoides*) in patients with haemoblastoses. n = number of cases, M = arithmetic means of bactericidin index values in the group, s = standard deviation of bactericidin index values. (The bactericidal index gives the ratio of microbe counts incubated with the serum for 4 h to the initial counts. The lower the bactericidal index, the higher the bactericidal capacity of the serum.) P_M = the significance of the differences in the means obtained in the diagnostic group as compared with the mean in controls, P_s = significance of the differences in standard deviations in the diagnostic group as compared with the standard deviation of the control group

	Controls	Acute leukaemia	Chronic myeloid leukaemia	Chronic lymphat. leukaemia	M. Hodgkin	Reticulo- and lymphosarcoma	Myeloma	Myelofibrosis
n	42	23	21	23	26	20	10	14
M	2.30	1.27	1.01	2.27	0.54	1.23	1.21	1.09
P_M		< 0.01	< 0.01		< 0.01	< 0.01	< 0.05	< 0.01
s	1.29	1.11	0.94	2.44	0.56	1.60	1.17	1.13
P_s				< 0.01	< 0.01			

Table II. Statistical evaluation of the values of bactericidal index *6 h* after incubation of the serum with microbes (*B. anthracoides*) in patients with haemoblastoses. n = number of cases, M = arithmetic means of bactericidin index values in the group, s = standard deviation of bactericidin index values. P_M = statistical significance of the differences in the means obtained in the diagnostic group as compared with the mean in controls, P_s = significance of the differences in standard deviation in the diagnostic group as compared with the standard deviation of the control group

	Controls	Acute leukaemia	Chronic myeloid leukaemia	Chronic lymphat. leukaemia	M. Hodgkin	Reticulo- and lymphosarcoma	Myeloma	Myelofibrosis
n	42	22	21	22	25	20	9	13
M	2.30	1.46	2.84	1.76	0.73	2.29	1.16	1.25
P_M		< 0.05			< 0.01		< 0.05	< 0.05
s	1.51	1.46	5.84	1.27	1.01	2.94	1.02	1.00
P_s			< 0.05		< 0.01	< 0.01		

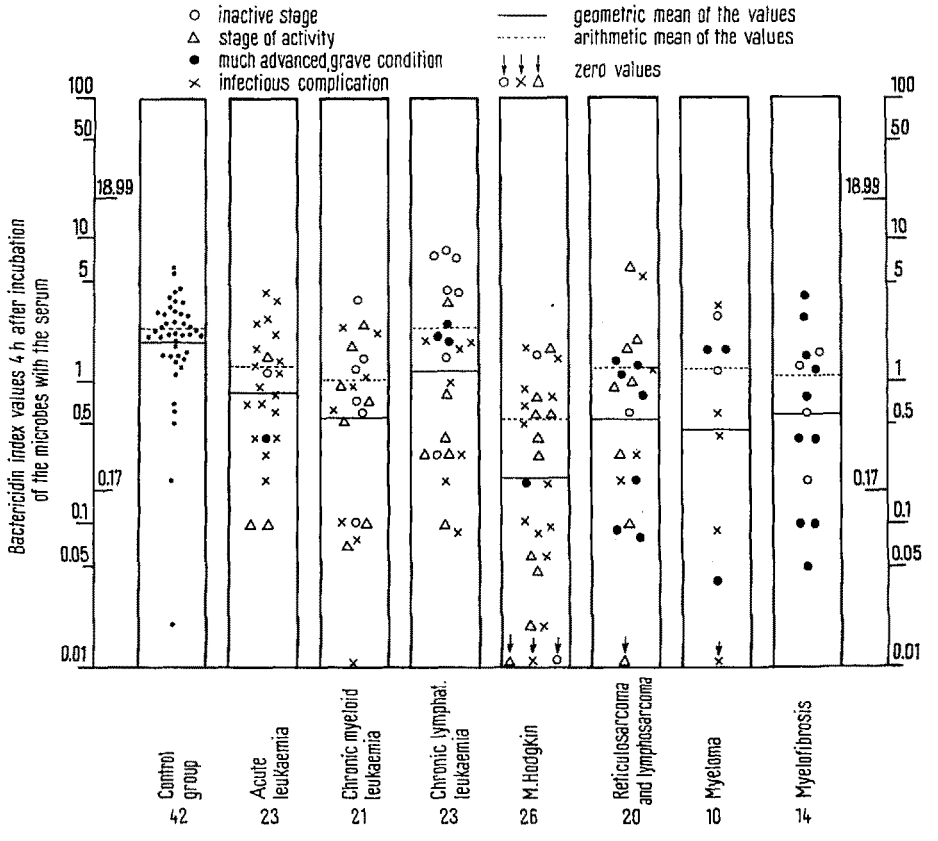

Fig. 1 illustrates the values of the bactericidin index *4 h* after incubation of the microbes (*B. anthracoides*) with the investigated sera in a control group of healthy subjects and in the individual diagnostic groups of haemoblastoses. The values presented in the Figure were obtained at the first examination only and do not include the results of repeat examinations. The figures given under the caption 'diagnoses' indicate the number of patients investigated in the group. The Figure also illustrates the degree of activity of the disease observed at the period at which the patients were investigated.

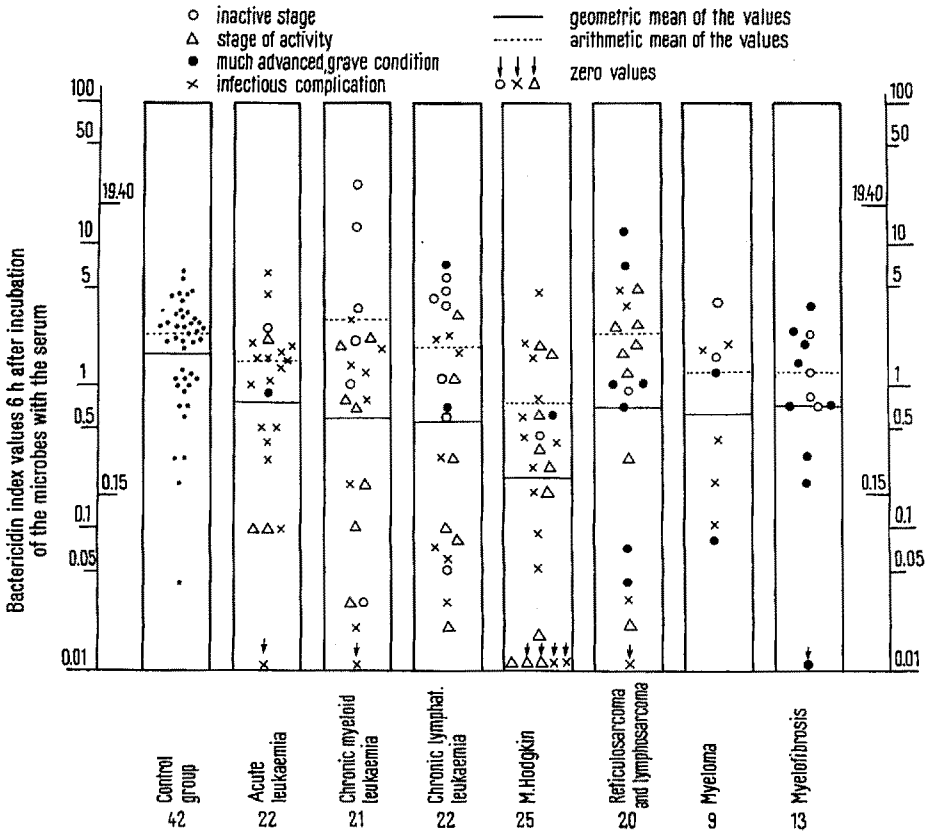

Fig. 2 illustrates the values of bactericidin index *6 h* after incubation of the microbes (*B. anthracoides*) with the investigated serum in a control group of healthy subjects, and in the individual diagnostic groups of haemoblastoses. The values presented in this Figure were obtained at the first investigation and do not include those of repeated examinations.

could be demonstrated. Only in chronic myeloid leukaemia, and especially in cases of Hodgkin's disease, the patients exhibiting high bactericidin levels suffered less from infectious complications than the patients of the same groups with normal bactericidin levels; in the lymphosarcoma and reticulosarcoma group, high bactericidin levels occurred mostly in patients with a more serious course and in a more advanced stage of the disease.

In all patients properdin and serum complement levels, as well as the capacity of antibody formation in response to a known antigen stimulus (brucella endotoxin), were concurrently investigated. The results were correlated with bactericidin levels; a relationship with properdin was found in the Hodgkin's disease group only. The relation was in a negative sense, i.e. patients exhibiting high mean values of bactericidin showed low mean properdin levels.

In comparing the bactericidal capacity of serum, it was necessary to decide whether the antibiotics applied to some of the patients with infectious complications do not influence the results. We have examined *in vitro* the sensitivity to antibiotics of *B. anthracoides*, which we were using as a testing microbe for the bactericidin estimation.

It could be shown that *B. anthracoides* was insensitive to penicillin, aureomycin and terramycin; it was little sensitive to chloramphenicol and well sensitive to streptomycin. The patients' analysis demonstrated that clinical doses of current antibiotics applied to 16 out of 65 cases with increased bactericidin values and to 28 out of 79 cases with normal values did not affect the bactericidal capacity of serum examined.

The significance of our findings of high bactericidin values in patients suffering from haemoblastoses is not easy to explain. On the basis of experimental studies it seems that bactericidin probably does not play an important role in effective immunity. This problem, however, is not quite clear. The correlation of bactericidin values with the clinical picture of our patients cannot explain this question either. We presume, however, that the role of bactericidin in immunity cannot be completely disregarded. The finding of ŠTERZL[5] showing that only the serum and not blood plasma possesses bactericidal activity *in vitro* does not seem to be a proof against the action of bactericidin *in vivo*, since in all inflammatory processes fibrin is formed, and thus a coagulation process with serum formation has taken place.

As it is known that higher bactericidin values can occur in the course of common bacterial infections, it would be possible to explain in this manner higher bactericidin values in those of our patients suffering from haemoblastoses complicated with associated infectious process. However, this cannot explain the finding of high bactericidin values found even in some of our patients, in whom an accompanying, complicating bacterial infection could not be proved clinically. Although we are aware of the fact that some complicating infectious processes may run a clinically latent course, we cannot consider this explanation to be sufficient, as the clinical signs of an infectious complication were very thoroughly searched for and the number of patients lacking these signs and showing high bactericidin serum levels was comparatively high.

However, the participation of other mechanisms cannot be ruled out, viz. extracts from leukocytes, the so-called leukins which also exert the bactericidal effect[4]. In extensive leukaemic infiltrations in various organs, a considerable number of leukocytes constantly undergoes disintegration and the leukins released may pass into the blood stream. A considerable leukocyte disintegration

occurs equally in common inflammatory processes, e.g. in pneumonia, where serum bactericidin level is high. Considering the fact that from the functional aspect leukins behave in the same way as does serum bactericidin, one cannot rule out the possibility that they may represent the same substance. Neither can it be ruled out that the bactericidin level may be raised by the haemoblastic process itself through a hitherto unknown mechanism.

Zusammenfassung. Die bakterizide Eigenschaft des Serums gegen grampositive Testbakterien *B. anthracoides* wurde an 136 Hämoblastosekranken untersucht. Bedeutend erhöhte Bakterizidinmittelwerte wurden bei akuten Leukämien, chronischen myeloiden Leukämien, M. Hodgkin, Retikulosarkom und Lymphosarkom, Myelom und Myelofibrose im Vergleich mit der gesunden Kontrollgruppe gefunden. Einzig bei chronischen lymphatischen Leukämien war der Bakterizidinmittelwert nicht erhöht.

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Effect of Ouabain on the Active Potassium Accumulation

Schatzmann[1] was the first to observe in 1953 that cardiac glycosides, even in the low concentration of 10^{-5}–$10^{-6}\,M$, inhibit the active cation transport of erythrocytes. A similar effect was found in other tissues, such as nerve[2], muscle[3], and ascites tumour cells[4] as well. At the same time, a number of authors stated that cardiac glycosides – while inhibiting the ion transport – do not in the least influence the carbohydrate metabolism of the cells[1,5]. This finding led to the conclusion that cardiac glycosides act directly on the carrier located in the cell membrane. This is supported by the fact that ouabain – the most effective of cardiac glycosides – is able to act in a very low concentration. $10^{-7}\,M$ ouabain exerts a 50% inhibition on the active ion transport, which seems to prove that the ouabain molecules directly block the active sites of the cell membrane involved in cation transport.

We tried to approach this transport inhibitory mechanism by experiments carried out on guinea-pig brain cortex slices and in human erythrocytes.

By our method described in previous papers[6,7], we incubated guinea-pig brain cortex slices, 100 mg wet weight per vessel at 37°C in air, by shaking in 2 ml of a standard medium containing $0.136\,M$ NaCl, $0.006\,M$ KCl and $0.03\,M$ tris HCl buffer pH 7.4. The incubation in the vessels was stopped at different intervals by treating the slices with trichloroacetic acid. The slices were then homogenized, centrifuged and washed, and the potassium content in the slices determined from the supernatant by flame-photometer. The analytical data were corrected for the swelling of the slices. Our results are shown in Figure 1.

If the brain cortex slices were incubated in a standard medium without substrate, they lost 60% of their K-content after the first 5 min of incubation, and on further incubation a continuous, slow K-outflow was evidenced. When after 5 min $0.02\,M$ glucose and $0.01\,M$ l-glutamate were added to the system, then an intense K-accumulation took the place of K-outflow. If, besides glucose and glutamate, $5 \cdot 10^{-6}\,M$ ouabain were added to the medium, the active K-accumulation was completely prevented; and instead a further strong K-outflow could be observed, which reduced the K-content in the slices to 10–15% of the original value.

The inhibition of K-accumulation in erythrocytes was investigated with ATP-rich erythrocyte ghosts prepared by our method[8]. Human red blood cells were partially haemolyzed in a 0.42 osmolar medium at 0°C, in this hypotonic state the cells were enriched with ATP, then after 2 min isotonicity was restored by 9% NaCl. These

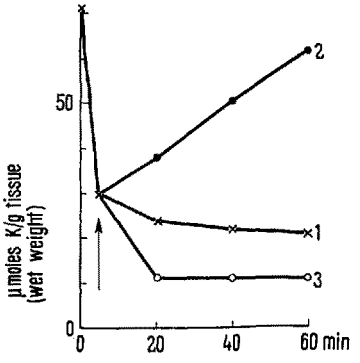

Fig. 1. Changes in the K content of brain cortex slices in the presence of ouabain at 37°C. 1 = Substrate-free control, $2 = 2.10^{-2}\,M$ glucose and $10^{-2}\,M$ l-glutamate, $3 = 2 \cdot 10^{-2}\,M$ glucose, $10^{-2}\,M$ l-glutamate and $5 \cdot 10^{-6}\,M$ ouabain. The substrates and ouabain were added at 5 min.

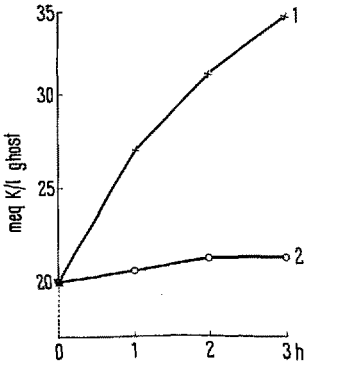

Fig. 2. The effect of ouabain on the K accumulation of ATP-rich erythrocyte ghosts at 37°C. 1 = Control, $2 = 10^{-5}\,M$ ouabain. The ATP content of the ghosts was 3400 µg/ml at 0 min.

[1] H. J. Schatzmann, Helv. physiol. Acta *11*, 346 (1953).
[2] P. C. Caldwell and R. D. Keynes, J. Physiol. *148*, 8P (1959).
[3] C. Edwards and E. J. Harris, J. Physiol. *135*, 567 (1957).
[4] M. Maizels, M. Remington, and R. Truscoe, J. Physiol. *140*, 61 (1958).
[5] H. A. Kunz and F. Sulser, Exper. *13*, 365 (1957).
[6] G. Gárdos, J. Neurochem. *5*, 199 (1960).
[7] G. Gárdos, Acta physiol. Acad. Sci. Hungar. *18*, 265 (1961).
[8] G. Gárdos, Acta physiol. Acad. Sci. Hungar. *6*, 191 (1954).