zwei bis drei Wochen eine 0,25%ige Maretinlösung, und zwar jeden 7. Tag 0,5 cm^3 intramuskulär.

Wie aus der Tabelle ersichtlich ist, wurde Butazolidin in täglichen Dosen von 20, 10 und 5 mg intramuskulär verabreicht. Alle angewendeten Dosen, auch diejenige von 5 mg, verursachten einen klar ersichtlichen Sturz der Leukozytenzahl. Wenn nach 14 Tagen, während welcher wir 10 und 5 mg verabreichten, die Medikation unterbrochen wurde, so war in den folgenden 14 Tagen eine weitere Leukozytenverminderung zu beobachten. Nach einem Monat erreichte die Leukozytenzahl wieder die Höhe, auf der sie sich vor der Butazolidinanwendung befand. Bei Hühnern, die 20 mg Butazolidin erhielten, haben wir die Dauer des Effektes nicht verfolgt. Es sei erwähnt, dass sich die Hühner während der Therapie normal verhielten, wobei Appetit und Eierlegen unverändert blieben. Im Differenzialblutbild waren keine besonderen Veränderungen festzustellen.

Aus diesen Untersuchungen scheint hervorzugehen, dass Butazolidin, zumindest bei Leukämien, nicht nur durch einen ausgeprägten antiphlogistischen und antipyretischen Effekt sowie eine eventuelle Wirkung über Hypophyse und Nebennierenrinde gekennzeichnet ist, sondern auch über einen direkten zytostatischen Effekt verfügt.

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Pharmakologisches Institut der Medizinischen Fakultät in Sarajevo, den 14. Juli 1954.

Summary

In leukosis of chickens caused by administration of 1-m-Tolylsemicarbazide (MARETIN-BAYER) Butazolidin caused a reduction of the number of leucocytes to normal values. This action might be attributed to a cytostatic effect of Butazolidin.

The Role of Amino-acids in Inducing Hormone Secretion

In a previous work we demonstrated[1] the role of anterior pituitary (via thyroid) in the deamination of amino-acids. Our further work started from the reversal of the question: instead of investigating the role of various hormones on the different phases of metabolism, the influence of different foods on hormone secretion was investigated. We supposed that if the pituitary has a role in protein metabolism, the amino-acids of the absorbed protein stimulate hormone secretion of the pituitary.

To study this assumption, the following experiments were carried out:

(1) *Eosinophile count.* (a) In men. We found eosinopenia in men 4 h after administration of the white of 5 boiled eggs or 1–2 g amino-acid (average change of − 31%), in contrast with the average increase of 18% in the fasting condition on the same subjects. In 3 Addisonian patients leucin had no eosinopenic effect (Fig. 1).

(b) In rats. 0·02 leucin, methionin, valin, glycin, phenylalanin, tryptophan, histidin or isoleucin were administered in 1 ml of 0·85% saline solution by stomach tube to normal fasting rats. Before, and 3 h after administration, eosinophiles were counted by the method of BACH and al.[1]. While in 20 out of 25 control animals (given saline solution), the eosinophile count decreased

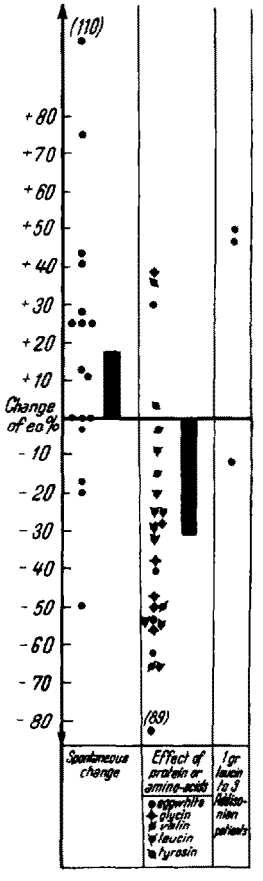

Fig. 1.–Effect of protein on eosinophile count.

less than 30%, we obtained very significant eosinopenia after the administration of leucin and methionin, and significant eosinopenia after the administration of valin, phenylalanin and tryptophan. Glycin, histidin and isoleucin produced no effect. In the statistical analysis, we used (WILCOXON method) the 5% significance level. On adrenalectomized rats 0·04 g leucin failed to cause eosinopenia (Fig. 2).

HARRIS and LANG[2] demonstrated the lypmphopenic effect of aspartic and glutaminic acid in rats. VARTIAINEN and APALAJAHTI[3] and HISSINK[4] found eosinopenia following administration of casein or tyrosin.

(2) *Effect of human serum on animals.* (a) On blood sugar: 2–3 ml serum taken before and 4 h after ingestion of the white of 5 boiled eggs, was injected to fasting rats. The blood sugar (HAGEDORN and JENSEN method) of rats receiving fasting serum showed slight decrease (average: − 8·4 mg%), the serum taken after a protein meal exerted a definite blood sugar raising effect (average: + 24 mg%). The difference between the two groups is

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[1] E. BACH, E. SZMUK, L. GYULAI, and A. VIRÁNYI, Orvosi Hetilap *92/35*, 1117 (1951).

[2] M. HARRIS and B. LANG, Proc. Soc. Exp. Biol. Med. *80*, 664 (1952).

[3] M. VARTIAINEN and J. APALAJAHTI, J. Clin. Endocrinol. Metab. *13*, 1502 (1953).

[4] G. HISSINK, Med. J. Australia *1953*, 631 and 746.

significant ("DD-method": $p = W_{16}[0{\cdot}11] < 0{\cdot}1\,\%$). The spontaneous change of the blood sugar of rats was on

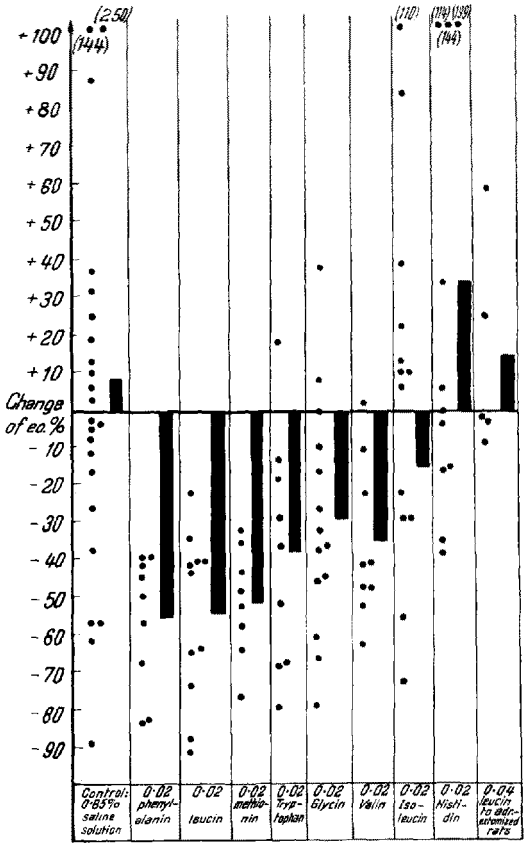

Fig. 2.–Effect of amino-acids on the eosinophile-count of rats.

the average $= 0$ mg%. Postprandial sera of patients with anterior hypopituitarism exerted no effect (Fig. 3).

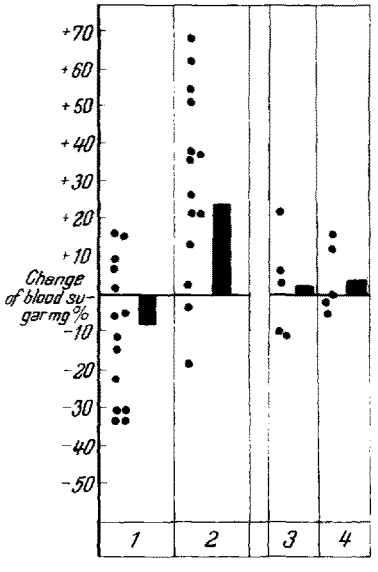

Fig. 3.–The effect of 2 ml human serum on the blood sugar level of fasted rats (injected s. c.):

(1) Sera of normal fasted subjects.
(2) Sera taken 4 h after the ingestion of 5 boiled eggwhites.
(3) Sera of fasted patients with anterior hypopituitarism.
(4) Postprandial sera of patients with anterior hypopituitarism.

(b) On eosinophile count. Sera taken similarly before and after protein or amino-acid (2 g glycin, valin, leucin or tyrosin) ingestion caused eosinopenia 3 h after injection to adrenal ectomized rats (average change: $-59\,\%$), while fasting sera caused no change (average: $+8\,\%$). The difference is significant ("DD-method": $p = W_{22}[0{\cdot}16] \ll 0{\cdot}1\,\%$). Sera taken from 3 patients with pituitary or adrenal insufficiency following protein meal, had no effect on the eosinophile count of rats (Fig. 4).

(3) *Ascorbic acid depletion test.* The intravenous administration of 0·04 g valin or leucin (neutralized to pH 7 with $NaHCO_3$) in saline solution caused depletion of ascorbic acid content 2½ h later in the adrenals of intact rats; pure saline solution had no effect. These findings are attributed to the mobilization of the rats' own ACTH from the pituitary (Table I).

Table I.–Ascorbic acid content of the rat adrenals (in γ/g adrenal weight) 2½ h after the intravenous injection of

0·85% saline	0·04 valin	0·04 leucin
360	238	261
361	257	270
385	281	288
405	288	306
451	331	338
479	346	340
490	396	378
492	410	428
504	Average: 318	Average: 328
505		
516	Average depletion: 146	Average depletion: 136
555		
572		
Average: 464		

(4) *Gonadotrophic effect.* 0·02 g of valin, leucin, tyrosin, methionin or glycin were injected subcutaneously in 1 ml 0·85% saline solution to infantile female rats (30–35 g) daily for 10–14 days. The rats were fed on a diet free of animal protein. The weight of the ovaries + uterus of the animals treated with amino-acids was significantly higher than that of the control group treated with saline solution. The ovaries of animals receiving amino-acids showed bleeding follicles and oedematous swelling of the uterus (Table II).

Table II.–Average weight of ovaria + uterus, removed from saline treated and amino-acid treated groups of infantile female rats:

	Animals	g
Control group	12	0·1071
Valin treated	5	0·2189
Leucin treated	5	0·1896
Tyrosin treated	5	0·2313
Methionin treated	5	0·2425
Glycin treated	5	0·1052

It is possible that one of the basal physiological impulses for hormone secretion is our daily food (not only proteins); eosinopenic effect of glucose, in larger amounts,

is well known[1]): The specific dynamic effect of foods can be explained by an acute pituitary stimulating effect.

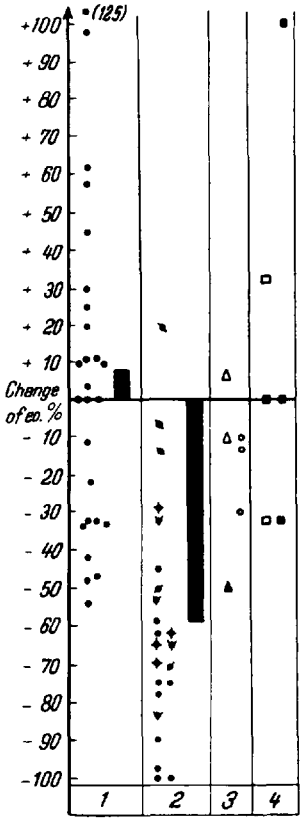

Fig. 1.–Effect of 2 ml human serum (injected s.c.) on the eosinophile-count of fasted rats:

(1) Sera of normal fasted subjects.

(2) Sera taken 4 h after the ingestion of 5 boiled eggwhites (●) or 1–2 g of: Glycin
Valin
Leucin
Tyrosin

(3) Sera taken after the ingestion of 2 g (△) or 100 g (▲) glucose, or 4 g butter (○).

(4) Postprandial sera of patients with Addison's resp. Simmonds' disease (■) compared with their fasted sera (□).

Perhaps the hormones of the blood stream are utilized for the metabolism of the absorbed foods and this is followed by hormone release in the pituitary.

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E. Bencze, C. Sávely, and
A. Majsay

Margit-Hospital, Budapest, May 17, 1954.

Zusammenfassung

Beim Menschen und in Tierversuchen wurde nachgewiesen, dass Eiweiss und verschiedene Aminosäuren Eosinopenie, Blutzuckererhöhung, Ascorbinsäure-Verminderung in den Nebennieren verursachen und bei infantilen weiblichen Ratten gonadotrope Wirkung ausüben. Es wird angenommen, dass die Aminosäuren der Nahrung einen physiologischen Impuls der Hormonsekretion darstellen und dass die spezifisch-dynamische Wirkung der Nährstoffe mit den obigen Befunden erklärlich ist.

[1] P. Constantinides, C. Fortier, and F. R. Skelton, Endocrinology *47*, 351 (1950). – J. Frey and E. Tecklenborg, Klinische Wschr. *30*, 516 (1952).

Detection of Antigens in the Embryo by Labelled Antisera

Coons and Kaplan[1] and Marshall[2] have shown that fluorescent labelled antibodies, applied to fresh frozen or dry frozen tissue, may reveal the localisation of antigens in the tissue. It seemed to us possible that the method might be well suited for the detection of tissue antigens during embryogeny or regeneration. The methods used by the above authors proved suitable when the antigens concerned were well characterised (pneumococcus, hormones, enzymes), while antisera prepared against tissue extracts may be expected to show a wide range of cross-reactions, and it therefore seemed desirable to modify the technique so that it would yield quantitative results. Antisera labelled with radioactive isotopes would seem to be suitable for this purpose. Pressman and Eisen[3] have used antisera labelled with I^{131} or S^{35} for the localisation of tissue antigens *in vivo*; and we have similarly used radioactive antisera, as histochemical reagents, combined with autoradiography, assuming that the density of grains on the film gives an indication of the relative concentrations or specificities of the antigens in different parts of the section.

Organ antisera, labelled by a modification of the method of Pressmann and Eisen with a solution of I_2 in KI^{131}, have been prepared for use on adult and embryonic stages of the house mouse, *Mus musculus*. The same material has also been studied[4] by means of fluorescent labelled antibodies, which might be expected to give better localisation within cells, although not suitable for quantitative estimations. Since some points of embryological interest have emerged, we have thought it appropriate to present a preliminary report; a full account will be published elsewhere.

A number of organ antisera have been made, and so far four of these have been studied; two anti-lens and two anti-cardiac muscle. Serum from a nonimmunized rabbit was used as a control. Both types of antisera showed some cross-reactions *in vitro* with extracts of non-homologous organs; anti-lens, for example, with skin and muscle extracts, anti-cardiac muscle strongly with skeletal muscle and more weakly with skin, liver and other organs. Antisera were applied to the sections both unabsorbed and after absorption with various tissues. The embryos to be investigated were frozen-dried, sectioned, and treated as recommended by Marshall except that, after treatment with the iodinated antisera, the slides were well washed and autoradiographs made as described by Doniach and Pelc[5]. Some sections were afterwards stained progressively with Mayer's haemalum.

Using *in vitro* immunological reactions, Ebert[6] has detected cardiac myosin in the primitive streak stage of the chick before there is any sign of the histological differentiation of the heart. We also find that antigens which react with anti-muscle sera can be detected in young embryos by an increased grain-density over regions of condensed mesenchyme in which histological differentiation has not yet occurred. For example, in a section of a limb of a 15–16 day old mouse embryo (Fig. 1), the areas occupied by the future limb muscula-

[1] A. H. Coons and M. H. Kaplan, J. exp. Med. *91*, 1 (1950).

[2] J. Marshall, J. exp. Med. *94*, 21 (1951); Exp. Cell. Res. *6*, 240 (1954).

[3] D. Pressman and A. N. Eisen, J. Immunol. *64*, 273 (1950).

[4] R. M. Clayton, Nature (in press).

[5] L. Doniach and S. R. Pelc, Brit. J. Radiol. *23*, 184 (1950).

[6] J. D. Ebert, Proc. Nat. Acad. Sci. *39*, 333 (1953).