

Zusammenfassung

Eine theoretische Betrachtung der Gleichungen von KLOTZ bestätigt folgendes: a) die O_2 -Aufnahme von Hämoglobin ist nicht nur durch statistische Faktoren bedingt; b) die Bindung der ersten O_2 -Moleküle erleichtert die Bindung weiterer Moleküle. Das steht in Übereinstimmung mit der Annahme von PAULING. Die physiologische Bedeutung dieses Phänomens wird graphisch dargestellt. Es wird eine Hypothese vorgeschlagen, die das Auftreten von hyperbolischen Dissoziationskurven unter bestimmten Bedingungen zu erklären vermag.

Zum Problem des Stoffwechsels der Brenztraubensäure *in vivo*¹

Die Brenztraubensäure (BTS) verdient unter den Zwischenprodukten des Kohlehydratstoffwechsels besonderes Interesse. Unsere Kenntnisse über ihre Umsetzung im lebenden Organismus sind lückenhaft, vor allem sind die Ansichten über die bei ihrer Verwertung beteiligten Hormone und Fermente widersprechend. Systematische Untersuchungen über den BTS-Haushalt beim normalen und beim alloxandiabetischen Kaninchen haben zu neuen Resultaten geführt, die im folgenden kurz zusammengefaßt sind.

1. Belastet man gesunde Kaninchen mit 0,25 g/kg Napyruvat intravenös, dann kommt es zu einem steilen Anstieg der Blut-BTS, der aber nur von kurzer Dauer ist. Nach 60 Minuten ist der Nüchternwert fast regelmäßig wieder erreicht. (Die Normalwerte der BTS für das Kaninchen liegen zwischen 0,5 und 2,5 mg %.) Eine längere Anstauung von BTS im Organismus wird also offenbar durch das Eingreifen von Regulatoren, die die BTS-Beseitigung steuern, vermieden.

2. Nimmt man eine gleich große BTS-Belastung bei einem Tier vor, bei dem durch Alloxaneinspritzung ein stationärer Diabetes erzeugt worden ist, dann unterscheidet sich die Blut-BTS-Kurve von der normalen durch einen meist noch höheren Anstieg und vor allem durch einen verzögerten Abfall zum Ausgangsspiegel. Nach 60 Minuten liegt die Blut-BTS regelmäßig über dem Nüchternwert, diesen oft um ein Mehrfaches übersteigend (Nüchternwerte beim stationären Alloxandibetes: zwischen 1,3 bis 4,3 mg % BTS). Beim Alloxandibetes besteht also eine eindeutige Verwertungsstörung für exogen zugeführte BTS.

3. Wird bei der Erzeugung des Diabetes eine höhere Alloxandosis verwendet, dann entwickelt sich beim Kaninchen ein Zustand von Glykosurie und Hyperglykämie progredienter Natur, d. h. die Tiere geraten unter fortlaufender Verschlechterung der Stoffwechsellage in einen dem menschlichen Coma diabeticum analogen Zustand mit stark verminderter Alkalireserve und Ketosis. Wir konnten nachweisen, daß bei diesem progredienten Typus des Alloxandibetes die endogene Bildung der BTS stark vermehrt ist. Die zunehmende Hyperpyruviämie ist für diese Diabetesform geradezu charakteristisch. An der Säurevergiftung des Organismus im Präkoma und Koma ist also die BTS in nennenswertem Maße beteiligt.

4. Angesichts der beiden Befunde von Verwertungsstörung exogen zugeführter und von Anstauung endogen gebildeter BTS erhebt sich die Frage nach den Regulatoren dieser Stoffwechselsubstanz, wobei der Gedanke an das Insulin am nächsten liegt. Entgegen verschied-

entlich in der Literatur beschriebenen Befunden haben wir keinerlei Beeinflussung des Blut-BTS-Gehaltes durch Insulin feststellen können. Der Nüchtern-BTS-Blutspiegel bleibt unbeeinflusst, der verzögerte Ablauf der Belastungskurve beim diabetischen Tier wird nicht verändert, die endogene Hyperpyruviämie im Präkoma und Koma reagiert nicht auf das Pankreashormon. Insulin ist also im BTS-Stoffwechsel kein Regulator.

5. Die Rolle des Vitamin B_1 als Bestandteil der Carboxylase ist aus Fermentarbeiten hinreichend bekannt. Das Vitamin liefert in Form der Aneurinpyrophosphorsäure ein Coferment, die sogenannte Copyrivodehydrase. Mangelzustände an Vitamin B_1 , wie sie bei der Beriberi vorliegen, führen zu erhöhtem Blut-BTS-Gehalt. Diese allgemein bekannten Tatsachen legen es nahe, die Einwirkung des Aneurins auf die pathologische BTS-Kurve des alloxandiabetischen Tieres im akuten Belastungsversuch zu prüfen. Das Ergebnis war eindeutig: es gelingt, die diabetische BTS-Kurve durch Zusatz von Aneurin zu normalisieren, d. h. der Ausgangswert für BTS wird nach 60 Minuten wie beim Normaltier erreicht, wenn man eine größere Aneurindosis $1\frac{1}{2}$ bis 2 Stunden vor der Pyruvatbelastung subkutan spritzt. Damit ist der Beweis für das akute Eingreifen des Aneurins in den BTS-Stoffwechsel erbracht.

Die Beseitigung der endogenen Hyperpyruviämie bei progredientem Alloxandibetes ist ein eigenes Problem. Im Zustand des Präkomas und Kommas handelt es sich um einen mehr oder weniger vollständigen Zusammenbruch der wichtigsten Regulatoren und ihrer Stoffwechselsubstrate, so daß die Wiederherstellung des Gleichgewichts nicht ohne weiteres von einem Angriffspunkt aus erreichbar ist. Es ist uns gelungen, auch diese Störung des BTS-Stoffwechsels zu kompensieren, worüber wir in einer nachfolgenden Mitteilung berichten werden.

S. MARKEES und F. W. MEYER

Medizinische Universitätspoliklinik Basel und Pharmakologisches Laboratorium der Firma F. Hoffmann-La Roche & Co. AG., Basel, den 5. Dezember 1947.

Summary

Following intravenous injection of pyruvic acid (PA) in the form of the sodium salt in the normal rabbit the level of PA in the blood rises, falling again to the initial level after 60 minutes. In the case of the alloxandibetic animal the elimination of PA administered in equal doses is markedly retarded. If animals are used that suffer from a progredient alloxan diabetes leading to death in coma, an increased endogenous hyperpyruviämia can be demonstrated. Insulin plays no part in the regulation of PA metabolism; through the application of aneurin, however, one succeeds in enabling the organism to make use of PA. A separate report will deal with the complex relations holding in endogenous hyperpyruviämia in coma.

Lethal Effect of Cosmic Ray Showers on the Progeny of Animals

The biologic effect of cosmic ray showers on the fertility of Angora rabbits and white mice was studied. Similar experiments with domestic rabbits were previously performed by J. EUGSTER and V. F. HESS¹ on the

¹ Der Großteil der Experimente wurde mit Unterstützung der Roche-Studienstiftung durchgeführt.

¹ J. EUGSTER and V. F. HESS, Die Weltraumstrahlung und ihre biologische Bedeutung (Verlag Orell Füssli, Zürich 1940), p. 139.

Hafelekar at a height of 2,340 m above sea-level. In view of the fact that in several points our findings differ from those of the aforementioned authors, it seems worth while to summarize briefly their results.

EUGSTER and HESS kept five test animals in cages covered with 18 mm of lead and four control animals within the same locality, but without lead covering. W4 bore without lead seven living young, whereas, when put under the influence of the showers, she remained sterile during six months, although repeatedly copulated. W2, kept for eight months under lead, remained sterile after repeated copulation; copulated without lead she gave birth to three living young, thereafter she was still kept for five months without lead. The autopsy revealed degeneration of the ovary. B2, after being kept for three months under lead, delivered eight dead, undeveloped embryos; after that she remained sterile for the following seven months, albeit repeatedly copulated. Histological analysis showed symptoms of cystic degeneration of the ovary. W1, after being kept for five months under lead delivered five dead, degenerated young, however, at autopsy her ovary proved to be normal. B1, kept for four months under lead, after being removed from the influence of the showers, remained sterile. The control animals were kept without lead: C2 bore four living young, and the autopsy of C3, C4, and C5 revealed normal ovaries. There is no indication to be found concerning the offspring of these last three animals. EUGSTER and HESS believe that cosmic ray showers produce in the females (according to histologic evidences in their ovaries) such permanent changes as result either in sterility or in inability to bear normally developed, healthy progeny. Apart from the small number of test animals—emphasized also by the authors—we should like to draw attention to several facts essential for the interpretation of the results.

First of all, the conditions in the altitude of Hafelekar were so abnormal that, judging from the losses in the animals' weights, an acclimatization of several months was necessary.

The inference that the degeneration in the ovaries of the animals is due to cosmic ray showers, i.e. connected with the reported lethal and sterilizing effects, is objectionable, because out of the three animals kept under lead and dissected, only two (W2, B2) showed such degenerations, and one of these (W2) was even able to deliver three well-developed living young, prior to the autopsy; the third animal (W1), which gave birth to degenerated and dead offspring, had a quite normal ovary. A permanent morbid change in the females is thus only supported by the lethal and sterilizing effects.

The lethal effect itself is questionable, on account of the circumstance that, in regard to two rabbits (B2, W1), it cannot be decided whether they were at all apt to have living progeny on the Hafelekar, and further, because the control experiments in which the birth of living offspring and the two still-births under lead occurred unfortunately were made at different seasons: the still-births happened in autumn, whereas the living young were delivered, except in the case of C2, in the spring.

On the other hand, the sterility found is rendered doubtful by the circumstance, known to every breeder, that the females are fertilizable only at certain periods (oestrus), which is recognizable by the swollen genitals marked with dark red veins; and further that even the males are not always inclined to copulation. In order to ensure copulation, it is customary to place the male previously at such a distance that he cannot smell the

scent of the female and to feed him with germinated rye or celery. After coupling the two animals the copulation must take place immediately, otherwise it is a sign that the animals are not in a suitable condition, and the act should be tried once more after a lapse of several days. These circumstances surely play an even more important role, if already the other conditions are abnormal. The fact that the animals were kept for several days in the same cage indicates that neither the oestrus of the females, nor the occurrence of copulation were sufficiently controlled; consequently, we are not faced in all the apparent cases of sterility with a real lack of fertility.

It seemed, therefore, worth while to reproduce the experiments, giving special care to the aforementioned circumstances. The experiments were performed in Budapest (110 m above sea-level) in an Angora rabbit farm under state control, where several hundred rabbits were bred. 16 mm of lead was placed above the one inch thick wooden cover of two of the usual cages, and the control animals were kept within the same locality, only at about 2 m distance from the lead layers. The control animals were always sister females, belonging to the same litter, and they were copulated with the same male as the test animals, with a delay of only one day; they gave birth in every case to normally developed young. The ensuing copulation was on all occasions watched personally by the head of the farm, who took also great care that the animals were fed with the same kind and quantity of fodder.

Experiments with Angora rabbits. Test animal No. 1 kept under lead was delivered after a normal gestation of 30 days of one living and three days later of six dead embryos, which perished at quite different stages of the embryonic evolution. The same test animal after a second copulation under lead remained sterile; when removed from the influence of the showers after copulating with the same male, she dropped a living litter of eight.

Test animal No. 2 begot without lead four living, thereafter, put under lead, she dropped five dead and again without lead eight living young.

Test animal No. 3 after copulating remained first, as well without lead as under lead, sterile, after a second copulation under lead she gave birth to nine dead young. Copulating again under lead and removed from the influence of the showers only for the day of the delivery, she gave birth to seven living young. One of these—which was thus kept, except on the day of its birth, constantly under lead—was used as

Test animal No. 4. It was delivered under lead of four dead and without lead of five living young.

Test animal No. 5 begot without lead five living young, after copulating and being kept for the whole time of its pregnancy without lead, was put under lead only the day of the delivery and dropped six dead young.

The results are summarized in the following table.

It should be stressed that, besides the test and control animals, twenty other rabbits were kept within the same locality; the fertility of all these animals proved to be normal, and they begot during the time of the experiments healthy, living offspring.

Out of the data collected in the table we may infer that neither sterility, nor a permanent change in the females, in any case not a change which would affect a later delivery, seems to prevail. As may be seen, out of the seven copulations under lead only in two cases sterility was observed, and the animals removed from the influence of the showers always delivered a living litter. Conversely, there seems to be good evidence for

	Without lead	16 mm Pb	Without lead
No. 1	—	1 living, 6 dead sterile	8 living
No. 2	4 living	5 dead	8 living
No. 3	sterile	sterile 9 dead	7 living (removed from lead for delivery) 5 living
No. 4	—	4 dead	
No. 5	5 living	6 dead (under Pb only for the delivery)	

the existence of a lethal effect, resulting most probably from the influence of cosmic ray showers upon the embryos. However, even these unambiguous results obtained in every single case investigated are not yet convincing. It seemed, therefore, necessary to investigate this lethal effect upon a greater number of animals, thus enabling a statistical check.

Experiments on white mice. The experiments were performed in the wooden tower laboratory of the institute. The animals were kept in a glass box of 60×130 cm basis and 40 cm height. Electric heating maintained a temperature of $25-30^\circ\text{C}$ within this box. The fresh air entered near the bottom of the box and could escape under the glass cover. The animals were installed immediately below the glass cover in 14 separate cages with aluminium walls, asbestos base, and covered with a wire net. Lead of 16 mm thickness was placed externally upon the glass box at a height of 10 cm above the animals.

First set: Here our aim was only to prove anew the effect of the showers. All the animals were kept under lead. 23 animals delivered in the course of three months 139 living, and 14 dead young; from these 139 animals 99 perished within a week after their birth. The mean loss was 82 ± 7 p.c.

The computation of the error was performed by reckoning for every animal separately the ratio of the still-births plus dead young to the sum of all the animals within the litter. The standard deviation from the mean value of the 23 cases were then computed for each individual case, and with the method of the least squares we thus determined the mean error of the mean value.

Second set: The lead was now removed from one half of the box, thus only about half of the animals were kept under lead, the other half, in quite similar conditions and upon identical diet, were kept without lead, but they were covered with a slate layer instead of lead in order to secure identical illumination. Within three months 21 animals kept under lead delivered 98 living and 4 dead offspring, besides two cases in which we were not able to count the number of the dead young. Out of the 98 living young 64 perished within a week after their birth, the mean loss being

$$71 \pm 6 \text{ p.c.}$$

This figure is in good agreement with that of the first set.

Conversely, out of the animals kept without lead, 16 delivered 93 living and 1 dead young in the course of the same three months. 23 of the 93 died within a week after their birth, the mean loss was:

$$22 \pm 9 \text{ p.c.}$$

The loss difference:

$$49 \pm 11 \text{ p.c.}$$

3 E. per.

represents the lethal effect due to the lead layer. The effect can be considered as real, since it is greater than four times its mean error. It is worthy of mention that, whereas all the rabbits begot under lead dead offspring, mice delivered mostly living progeny, which, however, perished within a short time after their birth. The birth rate was slightly higher without lead than with lead.

Since the intensity of cosmic ray corpuscles is practically the same with or without lead, the results, seem to indicate that the biologic effect must be attributed to the showers. (16 mm of lead is the most efficient thickness for generating cascade showers.) The difference in the biologic effect of the shower radiation compared to that of radioactive sources might be due to the following causes:

(1) From radio-active sources the heavily ionizing α - and β -rays are not able to penetrate into deeper layers of the body, and γ -rays or X-rays produce only about 100 ions per cm path in the body, hence, even when strong sources are used, ionization within a cell will not be very important. Conversely, a cosmic ray particle can penetrate through the whole body and produces there about 100,000 ions per cm of its path.

(2) The effect of radio-active radiation consists merely in ionization, a process which is reversible in consequence of recombination, whereas on account of their tremendous energies the cosmic ray particles can initiate irreversible nuclear transformations within a cell.

(3) In the core of a shower the particle density may be so great that a single cell is hit simultaneously by more than one ray.

It would be of interest to investigate whether the effect remains the same for showers created in iron instead of in lead; in which case it would be possible through magnetization of the iron to spread out the particles in the core of the shower over a wider area and consequently to examine the influence mentioned in point 3 separately. We should like to emphasize the importance of investigating with a sufficiently large number of individuals whether a permanent morbid change does occur in the females or not, and further whether the offspring born under lead are not more resistant against cosmic ray showers, i.e. whether a certain adaptation does not take place. Experiments with this purpose are now in progress.

Acknowledgements should be expressed to the head and owner of the Angora rabbit farm, Mrs. E. FABINYI, for much advice and extreme thoughtfulness, which were a great help in our Angora rabbit experiments.

J. BARNÓTHY and M. FORRÓ

Institute for Experimental Physics, University of Budapest, July 2, 1947.

Zusammenfassung

Angorakaninchen und weiße Mäuse wurden unter einer Bleischicht gezüchtet, deren Dicke (16 mm) die günstigste für die Auslösung von kosmischen Kaskadenschauern ist. Die Versuche ergaben einen Überschuss der Totgeburten und der Verluste innerhalb der ersten Woche von $49 \pm 11\%$ gegenüber den unter gleichen Bedingungen gehaltenen Kontrolltieren. Eine dauernde Beschädigung der Muttertiere sowie eine sterilisierende Wirkung scheint nicht vorzuliegen.

Urinary Excretion of Water-soluble Vitamins and Phosphorylation

Against the value of test doses applied for the estimation of vitamin deficiencies, the following objection can