These observations taken together with the results presented in this paper support the view that I is not merely a ganglion blocker with a uniform peroral absorption and a prolonged action due to its peculiar recirculation in the organism. (cf. BEYER *et al.*[1]).

Recent evidence[5] incidentally also points to the importance of central factors in the hypotensive action of ganglion-blocking agents representing several of the more 'orthodox' species known to possess such action.

Asa 214/1 was submitted to a brief clinical trial. 7 patients with grave fixed hypertension were treated for 8–33 days. The daily doses were from 15 to 90 mg. Asa 214/1 had a strong hypotensive effect, which, after a single dose, could last more than 12 h. The effect was practically the same after oral and subcutaneous administration. The effective dose was about 3 times as great as by treatment with I.

Parasympathetic side-effects were less pronounced than by treatment with I.

Asa 214/1, however, is unsuited for clinical use, as 5 of the treated patients in the course of 8–16 days displayed symptoms of cerebral disorder: tremor, ataxia and choreiform movements. Moreover, one patient was hallucinated and one developed a grave psychosis. All toxic symptoms disappeared in less than 30 days after discontinuation of the treatment.

Similar cerebral toxic symptoms have been described after treatment with I[6]. The cause of the symptoms is unknown.

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Zusammenfassung

5 Methyl-substituierte 3-Amino-norcamphane wurden im Vergleich zu 3-Methylamino-isocamphan in bezug auf Blockierung der Synapsen der autonomen Nervensysteme und blutdrucksenkenden Wirkung im narkotisierten Tier untersucht.

Die Ergebnisse deuten auf eine ganz spezifische Konstitutions- und Konfigurationsabhängigkeit der Wirkung sowie auf einen komplexen pharmakodynamischen Mechanismus, in dem die zentrale Blutdrucksenkung eine nicht unwesentliche Rolle spielt.

Erfahrungen aus kurzzeitigen klinischen Versuchen werden mitgeteilt.

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Survival of Bull Spermatozoa after Exposure to 150 atm N_2 for 15 Days

In the preservation of living cells through deep freezing, controllable factors are of interest. Among such factors pressure is one that can be easily and rapidly changed. LORANT, LORANT, ANGRIST, and KORPMAN[1], among others, have used high pressure as a means for storing blood at about – 12°C without freezing it. Investigations of high pressures in relation to freezing for the preservation of living cells are not to be found in the literature. MERYMAN[2] has studied blood preservation through deep freezing using blood protected by a moderate amount of dextrose which was sprayed through a nozzle against the surface of liquid nitrogen (– 197°C) and thus rapidly transferred into the frozen state. It has not been possible with MERYMAN's experimental conditions to achieve vitrification as investigated by LUYET[3], but the results when thawing and using the blood for transfusion are good and seem promising even for routine use. Further improvement of the method is thus of interest and could eventually be achieved if spraying was undertaken in an atmosphere under high pressure. Technically this would be possible using hydrogen or helium over liquid nitrogen.

Considering the practical value of vital deep freezing and the need to modify the process in different ways for different materials, it seems interesting to investigate the effect of high gaseous pressure on some living material of relevance in vital deep freezing. One such material is the living sperm cell. Earlier investigators[4] have demonstrated that mammalian tissues, such as skeletal muscle and heart muscle and also blood[5], can tolerate at room temperature hydraulic pressure of at least 1000 atm for short periods and for much longer periods if the temperature is lowered or the pressure not as high. These results are important if high pressure and release of the same is tried as a means of modifying the mechanics of freezing. They make it probable that the pressure itself would not be a first hand limiting factor. For our experiments bull sperm was chosen and was studied under pressure in a gaseous environment (packed in polyethylene tubes). Little or nothing seems to have been published concerning the tolerance of living mammalian cells against gases under high pressures. Our best knowledge seems to have been collected in deep diving and in such research the pressures hardly exeed 15 atm. A good tolerance, against higher gaseous pressures also, is evidently necessary if material for deep freezing is eventually going to be investigated and handled in atmospheres with a very much higher pressure.

As a first step in the investigation of preservative freezing under pressure, the author has tested living bull sperm with respect to the tolerance against two different gases, namely N_2 and H_2. A maximum pressure of 150 atm was used. Bull sperm was chosen because the preservative freezing of such material is already in routine use in some places and also because it is easily available from the farmers' organisations for artificial insemination. (For

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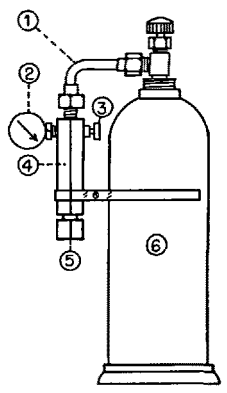
Two experiments were made in each group

Group No.	Exposuretime	Rate of pressure change	Initial motility %	Final motility %	Comments	Initial motility %	Final motility %
Nitrogen 150 atmospheres at + 6°C						Controls at atmospheric pressure and + 6°C	
1	20 min	Slow 15 s	50–60	50–60	Only few gas bubbles No damaged spermatozoa	50–60	50–60
2	20 min	Quick 2 s	60–70	60–70	as above (1)	60–70	60–70
3	36 h	Quick 2 s	50–60	45–55	Bubbles and foam. A few spermatozoa hurt with their tails lost	50–60	45–55
4	7 days	Quick 2 s	70–75	45–50	as above (3)	70–75	35–40
5	15 days	Quick 2 s	70–75	8–10	as above (3)	70–75	2–3
Hydrogen 100 atmospheres at + 6°C						Controls at atmospheric pressure and + 6°C	
6	7 days	Quick 2 s	45–50	2–3	Bubbles and foam.	45–50	8–10
7	11 days	Quick 2 s	45–50	a single per vision field	A few spermatozoa hurt with their tails lost as above (6)	45–50	2–3

recent literature on vital deep freezing of sperm and living tissue see[6].)

Sperm Preparation.—The sperm was prepared as follows. The ejaculate, which was collected at body temperature (+ 37°C), was diluted with a fluid containing 20 parts of egg yolk in 80 parts of a 2·9% Na-citrate solution with 0·1% streptomycin. This dilutent was also at body temperature. The suspension was slowly cooled down to about + 4°C at a rate not exceeding 0·5°C per min. The suspension was kept cool at + 4°C for 24 h and then used experimentally after the material had been judged and estimated with respect to viability. This estimation was done under the microscope on a heated table.



1 Connecting-tube. – *2* Manometer. – *3* Needle valve to release pressure and to control the rate of pressure change. – *4* Pressure chamber. – *5* Bottom-plug. – *6* Gas-cylinder.

Pressure Apparatus.—The pressure was obtained from a 7-m³ cylinder of compressed N_2 (150 atm) or H_2 (100 atm) which could be connected to a pressure chamber (see Figure). The chamber was made of 50 mm hexangular brass with a central bore of 10 mm. A threaded bottom-plug with high pressure packing permitted the opening and closing of the same when removing or introducing sample-tubes. A manometer to indicate the pressure and a needle valve to release the same, and also to control its rise and fall, completed the arrangement.

Sampling Technique.—The split sample method was used. Sperma suspension was drawn into a syringe in a cooling room at about + 6°C and put into thin polyethylene tubes of 4 mm internal and 4·5 mm external diameter. Each tube was 120 mm long. The tubes were first sealed at one end then filled and sealed also at the other by means of conical stoppers of ash wood. No air bubbles were left inside the tube. The stoppers were boiled for 1 h in distilled water. 8 filled tubes were used in each experiment and divided into 2 groups of 4. Of these one group served as control and was merely stored in the cooling room, whereas the other was put into the pressure chamber and exposed at cooling room temperature. The first experiments were made with N_2 at 150 atm. In later experiments H_2 at 100 atm was used because no other was available.

After each exposure 2 samples were taken out and their motility at + 37°C was compared microscopically with 2 samples from the controls. Except for this, all work was done in the cooling room at + 6°C.

In all, 7 different procedures were examined. Sperma suspension in polyethylene tubes was exposed to nitrogen at 150 atm for periods of 20 min, 36 h, 7 and 15 days. The effect of rapid (2 s) and slow (15 s) compression and decompression were also studied in the 20 min periods. During this short exposure the material was subjected to 3 serial compression-decompression cycles to test whether these repeated pressure changes would cause measurable damage.

2 experiments on the effect of hydrogen at 100 atm were made with exposure times of 7 and 11 days. In both cases pressures were applied and released in 2 s.

In every procedure the motility of the sperm was determined before and after exposure and compared with a control group at atmospheric pressure. Observations on the formation of bubbles or foam and also on mechani-

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cal damage of the spermatozoa were recorded in each case. The results are summarized in the Table.

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Zusammenfassung

Bullenspermien überleben in N_2 bei 150 atm und + 6°C gut 8 Tage (bis 14 Tage). In H_2 bei 100 atm und + 6°C nimmt die Überlebensfähigkeit ab. An den Spermien wurden Schäden durch Entwicklung von Gasbläschen bei Drucksenkung beobachtet. Weitere Versuche bei höherem Druck sind in reiner Flüssigkeitsumgebung auszuführen.

Die Wirkung von 4,6-Dinitro-o-kresol auf die Atmung eines experimentellen Rattentumors bei verschiedenen Temperaturen

Da sich die Wirkung von 4,6-Dinitro-o-kresol (DNOC) auf die Gewebsatmung des Warmblüters unter gleichzeitiger Berücksichtigung des Einflusses der Temperatur wesentlich von der auf die Gewebsatmung des Kaltblüters unterscheidet[1], andererseits aber auch am Tumorgewebe eine Aktivierung der Atmung mit Dinitrophenol-Verbindungen zu erzielen ist[2], dürfte auch an diesem Gewebe der Temperatur eine Bedeutung zukommen. Wir untersuchten daher den Einfluss von 10^{-5} M DNOC auf die *in vitro*-Atmung eines experimentellen Rattentumors, der durch subkutane Injektion von 9,10-Dimethyl-1,2-benzanthrazen erzeugt wurde und ein Spindelzell-Sarkom darstellte.

Der Temperaturbereich war 22,5–47,5°C. Atmungsmessung im indirekten Verfahren nach Warburg mit Krebs-Bicarbonat-Ringer (ohne Glucose!) als Medium. Versuchsdauer 3 h DNOC wurde entweder zu Versuchsbeginn oder nach 1 h zugesetzt. Sonstige methodische Einzelheiten wie in vorhergehenden Untersuchungen[3].

Es ergibt sich (Abb. 1), dass die Tumoratmung schon mit Temperaturerhöhung stark ansteigt, aber durch die angewandte DNOC-Konzentration noch weiter erhöht wird. Die Abbildung 2, welche die Umformung der Resultate in relative Aktivitäten bringt, lässt erkennen, dass diese Förderung systematisch mit Erhöhung der Temperatur erfolgt. Sie offenbart somit einen Verlauf, der im Gegensatz zu dem des Normalgewebes des homoiothermen Tieres, etwa der Leber der Maus[1], steht. Die %-Aktivitäts-Temperatur-Relation des Tumors entspricht daher dem Hemmtyp I nach Johnson[4], das heisst einer Bindung des Inhibitors bzw. Aktivators an aktives und denaturiertes Enzym und ergibt somit eine Beziehung, welche die Atmung der Leber von Kröten unter dem kombinierten Einfluss von DNOC und Temperatur zeigte und welche als für das Gewebe des poikilothermen Tieres typische angesehen werden konnte. Im Sinne der Theorie von Johnson muss demnach auch für das Tumorgewebe ein Enzymgleichgewicht von der Art angenommen werden, dass sich aktives und denaturiertes Enzym konzentrationsmässig nahestehen. Dadurch unterscheidet sich das Tumorgewebe wesentlich vom normalen Gewebe des homoiothermen Tieres, für welches eine extreme Verschiebung des Enzymgleichgewichtes zugunsten der aktiven Phase charakteristisch zu sein scheint[1]. Es geht offenbar die dem Tumor zugrundeliegende Korrelationsstörung so weit, dass der Tumor sozusagen poikilotherm im homoiothermen Organismus wird.

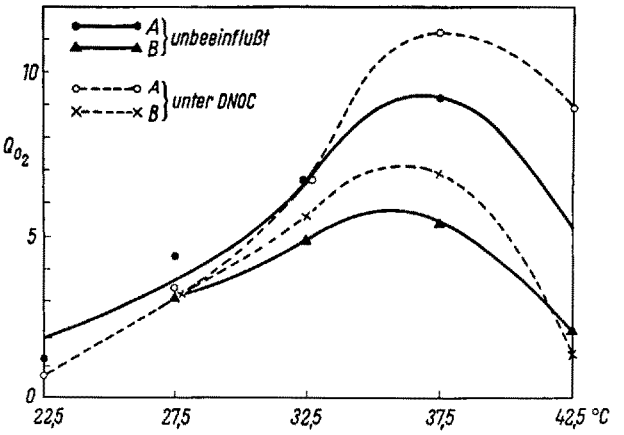

Abb. 1. Atmung des Ratten-Sarkoms unter Temperatur- und DNOC-Einfluss. *A* Q_{O_2} von 1.–3. h. *B* Q_{O_2} von 2.–3. h (bei DNOC-Zusatz 1 h nach Versuchsbeginn).

Eine weitgehende Übereinstimmung der DNOC- und Temperaturwirkung auf Kaltblüter- und Tumorgewebe besteht auch darin, dass sich beim Tumor die Aktivierungsenergie (μ) der Atmung gleichfalls erhöht: Bei DNOC von Anfang: μ = 18 300 → 27 800 cal, bei DNOC nach 1 h: 14 500 → 18 700 cal.

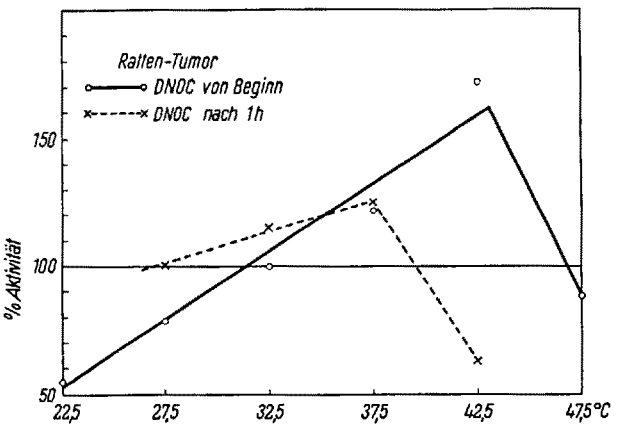

Abb. 2. %-Aktivität der Atmung des Rattentumors unter Einfluss von DNOC bei verschiedenen Temperaturen.

Für eine Übereinstimmung der morphologischen Organisation des Tumorgewebes des Warmblüters mit Geweben poikilothermer Tiere sprechen neue Befunde von Elias[5].

Trotz der weitgehenden Analogie in den Befunden von Tumor- und Kaltblütergewebe bleibt es aber fraglich, ob die Entsprechung für Tumorgewebe überhaupt gilt oder auf unseren Experimentaltumor, der eine für Tumorgewebe ungewöhnlich hohe Atmung zeigte, beschränkt werden muss.

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