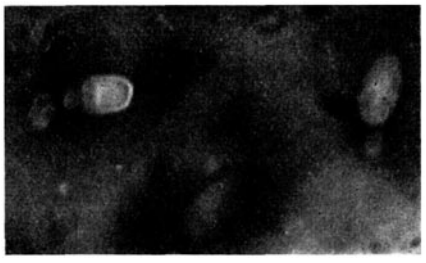
An Abnormality of Mammalian Spermatozoa

LAGERLOF, MCKENZIE and BERLINER, SWANSON and HERMAN, GUNN et al., WILLIAMS, HAQ, BLOM[1] and others have described various types of abnormal sperms in a number of species of animals. It appears that the following sperm abnormality, observed in the rabbit semen, has not been described so far.



Microscopic examination of the semen samples collected by means of an artificial vagina from the buck rabbits, showed that a small percentage of the sperm heads had protoplasmic masses instead of tails (fig. 1). On histological examination, a large number of the seminiferous tubules of the testes showed arrested spermatogenesis while others showed marked atrophic and degenerative changes. It appears that this abnormality was associated with defective spermatogenesis which ultimately resulted in the arrested development of the sperm tails. These inbred buck rabbits were classed as infertile in view of their poor breeding records. It was interesting to find that thyroid therapy stimulated spermatogenesis and greatly improved the semen quality of the treated buck rabbits. Detailed results will be described elsewhere. M. MAQSOOD

School of Agriculture, University of Cambridge, England, March 5, 1951.

Zusammenfassung

Es wird eine morphologische Abnormität der Kaninchenspermien beschrieben: Anstelle des Schwanzes befindet sich hinter dem Samenkopf eine protoplasmatische Masse. Diese Abnormität wurde am Samen von Kaninchen mit sehr schlechtem Fruchtbarkeitsgrad beobachtet. Mit Schilddrüsentherapie konnte die Samenqualität der betreffenden Kaninchen im Vergleich zu Kontrolltieren bedeutend verbessert werden.

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Use of Track Autoradiography in Studies on the Sulfur Metabolism of Connective Tissue

Preliminary Results obtained on Adult Cartilage

The problem of the fate of inorganic sulfate administered to animal tissues has been dealt with by some workers during the last decade. BORSOOK *et al.*[1] using labelled sulfate in the human body were the first to propose an exchange between inorganic sulfate administered and tissue sulfate. Further studies by SINGHER *et al.*[1] on rats showed that the radioactive sulfate given was held mainly by bone and bone marrow. These findings were confirmed by DZIEWIATKOWSKI[2], and the same author (DZIEWIATKOWSKI *et al.*[3]) reported that suckling rats given labelled sulfate sulfur retain S^{35} in articular cartilage, and it is suggested that the $S^{35}O_4$-ion is used in the synthesis of chondroitin sulfate. LAYTON *et al.*[4], injecting pregnant rats with labelled sodium sulfate, were able to show that the radioactive material had crossed the placental barrier and was fixed in mesenchymal tissues of the embryos. The same authors[5] cultured tissues from chicken embryos of varying age on a medium containing carrier-free S^{35} as SO_4-ions, and found a fixation of the isotope in various tissues.

To sum up: the investigations hitherto performed have shown that inorganic sulfate sulfur administered is partly utilized in the tissues. These results, however, are mainly based on studies on embryonic or growing tissues. But it may be supposed that even adult cartilage has a certain rate of sulfate ion fixation. Cartilagineous structures would therefore be suitable for autoradiographical studies. No investigations on S^{35} adopting the autoradiographic technique have been published so far as we know. The new method of track autoradiography, which has been developed recently, gives high sensitivity and efficiency due to use of highly sensitive nuclear track emulsions. As S^{35} has a very low β-ray energy, 0·169 MeV, this isotope is very suitable for this type of autoradiography[6]. Useful data regarding the more exact topographical determinations of the labelled sulfate ions might thus be obtained by using this technique.

The present paper deals with the uptake of labelled sulfate ions given as $Na_2S^{35}O_4$ in catilagineous tissues of adult rats by means of track autoradiography.

Experimental

Adult albino rats were given carrier-free $Na_2S^{35}O_4$[7] by intraperitoneal injections in the form of a single dose of $7{\cdot}0 \cdot 10^6$ counts/min/100 gm body weight. A total of 19 rats were used.

All the animals were placed in individual wire cages and given a standard diet and water *ad lib.*

The rats were killed 21, 49, 54, 73 and 77 hours after the administration of the labelled sulfate. Blood samples were taken by puncture of the heart under ether anesthesia immediately before death. The tissues to be examined were taken out quickly after death and washed in physiological saline. Some nasal septa were taken for activity determinations by the use of a 2·4 mg per cm^2 mica end-window Geiger-Müller tube. The blood was pipetted directly into the measuring cup, haemolysed, dried and measured. A correction curve was made to get the self-absorbtion of the sample. The nasal septa were cleaned from the mucous coat, re-washed in physiological saline, and dried flattened out on mica sheets. After drying, thin areas were trimmed off. The

[1] H. BORSOOK, G. KEIGHLEY, D. M. YOST, and E. MCMILLAN, Science *86*, 525 (1937).

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[2] D. D. DZIEWIATKOWSKI, J. Biol. Chem. *178*, 197 (1949).

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[4] L. L. LAYTON, DORIS R. FRANKEL, and SYLVIA SCAPA, Arch. Biochem. *28*, 142 (1950).

[5] L. L. LAYTON, DORIS R. FRANKEL, and SYLVIA SCAPA, Cancer *3*, 275 (1950).

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[7] Obtained from AERE, Harwell, England.

septa are regarded as "infinitely thick sources" and corrections for self-absorbtion were obtained in accordance with the method of ATEN[1]. The activity was measured with and without absorber, the dried cartilage weighed and the area measured in a projection apparatus.

Samples of trachea, proc. ensiformis, ear, nasal septum and cornea were frozen in liquid air and dehydrated in chilled and dried alcohol at −35°C and embedded in paraffin *in vacuo* according to a method worked out by one of us[2].

Sections cut at 10 μ were floated on nuclear track plates (Kodak *NT* 4), deparaffinized, exposed for 48 hours and developed. To get rid of accumulated cosmic ray tracks, the plates were eradicated in humid air immediately before use. During exposure the plates were placed in a cosmic ray shield. After the developing procedure the sections were stained in 0·01 per cent Azur I in 20 per cent alcohol and mounted in Permount.

The autoradiograms were studied in the microscope using a 60× apochromatic objective and a 15× compensation eye piece with a square-net placed in the diaphragm which limited an area of 1500 μ^2 at the magnification used. To get data on the distribution of the tracks in the different structures, countings were performed with the aid of the square-net.

Thus 20 countings were made with the square-net area within the central part of the tracheal cartilage. For comparison similar countings were performed within the connective tissue structures of the mucous coat of the trachea, and within the emulsion beside the section. Four rats were used for such determinations. Two of them were killed 54 hours and the other two 77 hours after injection of the labelled sulfate.

Results and discussion

Of the 19 rats used in these experiments, 15 were used for activity measurements of blood and nasal septum in the Geiger-Müller counter. Blood activity seems to be obtained with sufficient accuracy (10%), considering the great individual variations, by measuring directly on the dried sample. The dried flat nasal septum is a rather homogeneous structure with an area of 25 to 40 mm^2 and about 100 microns in thickness. A direct activity measurement on such samples is naturally rather rough. With proper corrections, as outlined by ATEN[1], however, the accuracy seems to be quite sufficient. The results of these measurements are shown in table I.

Table I

Group	No. of animals	Hours after injection	Counts/min/gm (M)	
			Blood	Cartilage
I	5	21	3,000	52,000
II	5	49	1,300	78,000
III	5	73	140	8,200

The figures given are mean values of five rats in each group. From this table it is seen that the concentration of S^{35} in the blood falls rapidly during a relatively short time. This is in accordance with the results of DZIEWIATKOWSKI. In contrast to the rapid fall of the activity in blood, a pronounced rise is found in the cartilage of the nasal septum followed by a decrease of activity after 49 hours. Comparing the figures of activity of the tissue examined by DZIEWIATKOWSKI with our figures on nasal septa, it is obvious that the value of specific activity in the cartilage examined by us is greater than in any other tissue. These results give evidence of a high rate of incorporation of radioactive sulfate ions even in adult cartilage. The high specific activity for bone marrow and extract of bone marrow reported by DZIEWIATKOWSKI may be explained as being due to contamination of the marrow with cartilagineous tissue.

Table II

Animal	Hours after injection of S^{35}	Number of tracks ($M \pm \varepsilon$) in		
		cartilage	mucosa	background
1	54	10·7 ± 2·7	2·8 ± 1·3	0·6 ± 0·2
2	54	9·1 ± 1·4	1·5 ± 0·9	0·3 ± 0·1
3	77	8·9 ± 0·6	2·3 ± 0·3	0·4 ± 0·1
4	77	6·7 ± 1·7	0·7 ± 0·9	0·1 ± 0·1

The autoradiograms obtained were satisfactory. The sections showed well preserved tissue which stained quite well. The metachromatic tint of the cartilage made an orientation within the sections very simple. By proper handling of the slides we got practically unstained emulsions, which were perfectly translucent with few fog grains. The tracks of the β-rays were easily recognized built up by densily exposed, closed packed grains (see figure). The tracks were conveniently separated from each other and the starting points could mostly be determined exactly.

Rat trachea showing numerous tracks in the cartilage and a few in the connective tissue of the mucosa (M). Background fog without any tracks in the upper right corner (250 ×).

The results obtained by counting the tracks on the autoradiograms are given in table II for the cartilage and mucosa of trachea of four rats.

From this table it is evident that the number of tracks is very high in the tracheal cartilage as compared

[1] A. H. W. ATEN, Nucleonics *6*, 68 (1950).
[2] B. H. PERSSON (unpublished results).

with the mucosa. Very few tracks were observed in the emulsion beside the sections. This background is due to cosmic rays, which reach the plate during exposure in spite of the shield.

The typical distribution of the tracks is clearly seen in the photomicrographs (fig. 1), which show the section lying over the emulsion.

The results obtained in this work show that the method of track autoradiography may be capable of giving quantitative values of activity of S^{35} in tissue structures even within a minute volume of the section. In the present preliminary work, the capacity of the method has of course not been fully utilized in this respect. Further studies with S^{35} are proceeding and possible improvements of the track autoradiographical method are being searched for.

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Department of Histology, University of Upsala, January 15, 1951.

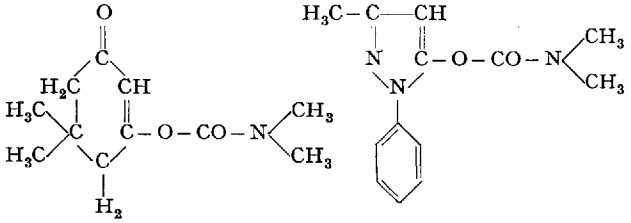
Zusammenfassung

Die Fixierung von radioaktiven Sulfationen in ausgewachsenem Bindegewebe von Ratten wurde mit Hilfe von Spurautoradiographie studiert. Die Autoradiogramme zeigen eine deutliche Konzentration von S^{35} im Knorpel und verhältnismäßig wenige Spuren in anderen Bindegeweben. Die angewandte autoradiographische Methode ermöglicht die Bestimmung der Aktivität von S^{35} innerhalb sehr kleiner Gewebegebiete.

Zur Spezifität sogenannter Esterasegifte

Die Hemmung der zentralen und peripheren Cholinesterasen durch Diäthyl-*p*-nitrophenyl-thiophosphat (Parathion) wurde erstmals von DUBOIS und Mitarbeitern[1] in einer grundlegenden Arbeit beschrieben. Im Säugerorganismus zeigt dieses Insektizid einen cholinergischen Effekt und bewirkt bei höheren Dosen die für eine Erregung des Parasympathikus bzw. Vagus durch Azetylcholin typischen Symptome. Noch stärker treten die Wirkungen bei dem Sauerstoffanalogen Mintacol[2] in Erscheinung, das als Miotikum und Mittel zur Glaukombehandlung empfohlen worden ist. Eine intensive Hemmung der Fermentaktivität durch Parathion und verwandte Verbindungen wurde auch bei der aus Nervengewebe von Insekten gewonnenen Cholinesterase von METCALF und MARCH[3] beobachtet. Die beim Insekt auftretenden toxischen Symptome stehen in einer direkten Beziehung zum Ausmaß der Esterasehemmung; bei Eintritt des Todes wurde eine vollständige Blockierung des Enzyms festgestellt. Der Schluß erscheint berechtigt, daß diese Fermente den entscheidenden Angriffspunkt des Parathions bilden und daß somit ein ähnlicher Wirkungsmechanismus vorliegt wie bei den auch chemisch verwandten Kampfstoffen der Phosphorsäuregruppe und weiterhin bei DFP, HETP und TEPP.

In den Forschungslaboratorien der I. R. Geigy AG., Basel, sind in neuester Zeit weitere hochwirksame Insektizide entwickelt worden, die chemisch einer ganz anderen Körperklasse angehören. Unter diesen Verbindungen beanspruchen die durch GYSIN dargestellten Urethane G 19258 [1] und G 22008 [2] ein besonderes Interesse.



G 19258 G 22008

Bei der Untersuchung dieser Verbindungen auf eine Inhibitorwirkung gegenüber Azetylcholinesterase wurde die Spezifität dieser Wirkung abgeklärt im Vergleich zum Effekt auf andere esterspaltende Fermente. Wir prüften die Wirkung der Präparate auf die Novocain-[3] und Cholinesterasen des menschlichen Blutes, ferner auf die Parpanitesterase[4] des Kaninchenplasmas. Die Bestimmung der Aktivität der azetylcholinspaltenden Fermente erfolgte bei einer Substratkonzentration von 0,0066 m nach der manometrischen Methode von AMMON[5], wobei die Pseudocholinesterase des Serums und die Cholinesterase aus hämolysierten Blutkörperchen getrennt zur Untersuchung kamen. Die Aktivität der Parpanitesterase wurde nach der von uns publizierten Arbeitsvorschrift[4] bestimmt. Mit gleicher Versuchsanordnung studierten wir auch das Verhalten der neuen Insektizide gegenüber der Novocainesterase. Die Substratkonzentration betrug hier 2 mg%, die analytische Bestimmung des Novocains erfolgte entgegen der zitierten Originalvorschrift[4] durch Extraktion mit Äthylendichlorid nach Bikarbonatzusatz, wobei das Pharmakon von seinem Spaltprodukt Diäthylaminoäthanol quantitativ abgetrennt werden kann. Bei den Hemmungsversuchen mit Eserin müssen die durch diese Base bedingten niedrigen Blindwerte bei allen Messungen berücksichtigt werden.

Als Vergleichspräparate haben wir das für Insekten ungiftige Parasympathikomimetikum Eserin sowie das Parathion, als typischen Vertreter der Insektizide aus der Gruppe von Phosphorsäureestern, in die Untersuchungen einbezogen. Für jedes Präparat wurde der Inhibitoreffekt bei verschiedenen Verdünnungen ge-

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[5] R. AMMON, Arch. ges. Physiol. *233*, 468 (1933).

	Konzentrationen, welche die Aktivität um 50% hemmen			
	G 19258	G 22008	Parathion	Eserin
Cholinesterase	$2{,}0 \cdot 10^{-4}$ m	$1{,}2 \cdot 10^{-5}$ m	$1{,}5 \cdot 10^{-3}$ m	$6{,}7 \cdot 10^{-7}$ m
Pseudocholinesterase	$1{,}6 \cdot 10^{-5}$ m	$6{,}1 \cdot 10^{-7}$ m	$9{,}6 \cdot 10^{-5}$ m	$2{,}8 \cdot 10^{-7}$ m
Parpanitesterase	$4{,}5 \cdot 10^{-8}$ m	$3{,}6 \cdot 10^{-9}$ m	$1{,}9 \cdot 10^{-5}$ m	$>0{,}3 \cdot 10^{-4}$ m
Novocainesterase	$7{,}5 \cdot 10^{-6}$ m	$2{,}0 \cdot 10^{-7}$ m	$6{,}0 \cdot 10^{-6}$ m	$1{,}5 \cdot 10^{-7}$ m