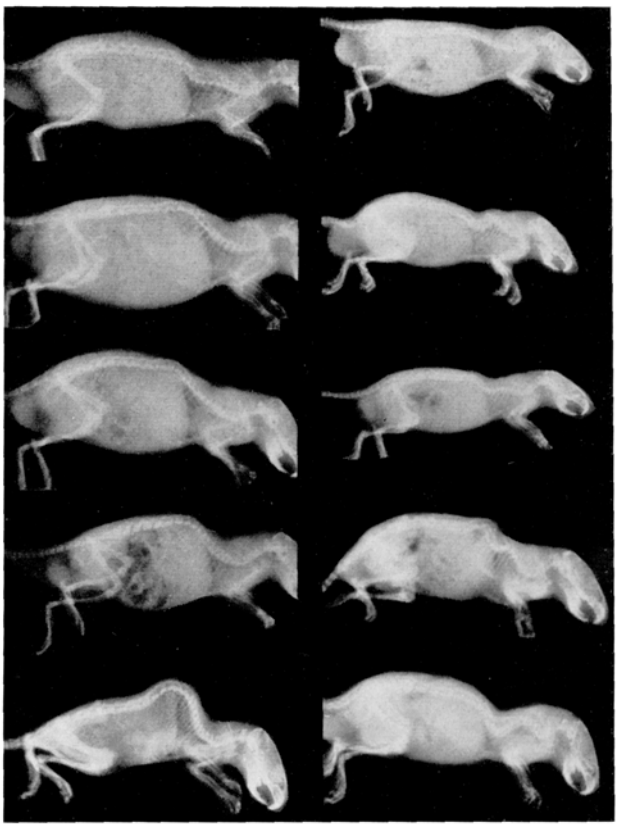
give rise to spinal curvatures similar to those produced by BAPN (Figure).



X-ray photographs of rat spines. Right-hand column, top to bottom: normal; 0·175% BAPN·H·Fumarate for 37 days; 0·3% cystamine·2HCl for 37 days; 0·2% semicarbazide·HCl for 41 days; 0·1% acetone semicarbazone for 31 days. Left-hand column, top to bottom: 0·1% *p*-hydrazinobenzoic acid for 31 days; 1·0% 4,4-diphenylsemicarbazide for 44 days; 0·2% 1,5-diphenyl carbazide for 49 days; 0·1% 1,3-diethyl-2-thiourea for 49 days; 0·1% thiosemicarbazide for 31 days.

We have also produced kyphosis by feeding the following dietary levels of various chemicals to rats weighing 50–60 g (Figure): 0·1% *p*-hydrazinobenzoic acid, 1·0% 4,4-diphenylsemicarbazide, 0·2% 1,5-diphenylcarbazide, 0·1% 1,3-diethyl-2-thiourea, and 0·1% thiosemicarbazide. Of these substances, *p*-hydrazinobenzoic acid caused spinal curvatures most similar to those produced by BAPN. Thoracic cage deformities were also present, but exostosis formation on mandibles and femurs was slight.

The spinal curvatures caused by 4,4-diphenylsemicarbazide, 1,5-diphenylcarbazide, and 1,3-diethyl-2-thiourea were less severe, but definitely palpable after 3–5 weeks of feeding. No gross evidence of exostosis formation was seen in these animals. The diphenylcarbazide, however, gave rise to marked *splenomegaly* and *anemia*.

The spinal curvature produced by thiosemicarbazide was different in character from that of osteolathyrism (Figure). This was probably due to the occurrence in these animals of a very *severe atrophy of the muscles* and a shortening of the tissue elements surrounding the spine. That thiosemicarbazide toxicity is probably related to BAPN toxicity is evidenced by the fact that *damage to the aortic media* occurred in these animals (MILLISER and DASLER: unpublished). Some exostosis formation on the medial aspects of the femurs was also evident grossly.

We have endeavoured to find a common denominator for the similar effects on skeleton and aorta of such dissimilar chemicals as BAPN, mercaptoethylamine, and semicarbazide. Much of the senior author's earlier work on protective agents against sweet pea and BAPN toxicity[10,3] was based on the premise that BAPN acted as a competitive antimetabolite. This possibility now appears remote. We now feel that coordination complex- or chelate-formation may be the common mechanism by means of which these substances induce similar toxic effects. All of these substances do form chelates. Thus far, however, we have been unable either to prove or to disprove this hypothesis. The feeding of disodium (ethylene-dinitrilo) tetraacetate (EDTA) at levels of 0·5% of the diet neither accelerated nor delayed the progress of the disease in BAPN-fed rats.

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Zusammenfassung

Starke Kyphoskoliosen werden in Ratten durch Fütterung von osteolathyrogenischen Substanzen wie 2-Aminopropionitril, Aminoacetonitril, 2-Mercaptoethylamin, Cystamin, Semicarbazid und Acetonsemicarbazid induziert. Kyphosen können auch durch *p*-Hydrazinobenzoesäure, 4,4-Diphenylsemicarbazid, 1,5-Diphenylcarbazid, 1,3-Diäthyl-2-thioharnstoff und Thiosemicarbazid hervorgerufen werden. Es wird vermutet, dass derselbe toxische Mechanismus dieser chemisch ungleichen Substanzen durch Bildung von Koordinationsverbindungen oder Chelaten bedingt ist.

[10] W. DASLER, Proc. Soc. exp. Biol. Med., N. Y. *91*, 554 (1956).

The Effect of Lipid Emulsions on the Blood Lipoproteins of Atheromatous Rabbits

In the study of the effect of glucocorticoids on experimental rabbit atheromatosis, it was found a retrogression of aortic atheromatosis in rabbits receiving a phospholipid emulsion, whereas upon neutral fat infusions no such changes in atheromatosis could be observed[1].

In the present work it was intended to ascertain (1) whether atheromatous rabbits, after infusions of lipid emulsions, show changes in blood lipoproteins as well as changes in blood lipids, (2) in what respect would changes in the blood lipoprotein spectrum affect the properties of cholesterol of the lipoproteins, (3) relationship of such changes to the retrogression of the atheromatous process. Atheromatous rabbits were given infusions of emulsified neutral fat containing olive oil (7%), Tween 80 (0·6%), and monoglyceride K (1%). The phospholipid emulsion contained crude soya lecithin (4%). Control rabbits received stabilisers in 5% glucose or glucose alone. The duration of the individual infusions was 9–10 h. Lipoprotein changes were studied by paper electrophoresis. The individual fractions were analysed for cholesterol

[1] V. FELT, D. REICHL, S. ROEHLING, and S. VOHNOUT, Gerontologia, in press.

Table

Infusion liquid	Rabbit No.	Before infusion cholesterol in			After infusion cholesterol in		
		α-globulin	β-globulin	α-globulin / β-globulin	α-globulin	β-globulin	α-globulin / β-globulin
Stabiliser or 5% glucose (group 1)	1	26·5	154·2	0·17	45·3	117·6	0·38
	2	16·3	385·5	0·04	52·0	327·6	0·15
	3	11·0	76·0	0·14	6·2	35·0	0·18
	4	10·6	110·8	0·09	8·6	132·5	0·06
Neutral fat emulsion (group 2)	5	25·3	56·3	0·44	62·3	56·3	1·11
	6	8·7	138·4	0·06	101·7	87·2	1·22
	7	152·7	263·5	0·57	227·2	208·0	1·09
	8	24·6	52·2	0·47	67·0	67·0	1·00
	9	31·7	309·5	0·10	235·5	169·9	1·40
Phospholipid emulsion (group 3)	10	21·0	434·0	0·04	219·5	134·2	1·63
	11	18·3	172·0	0·10	113·2	43·9	2·58

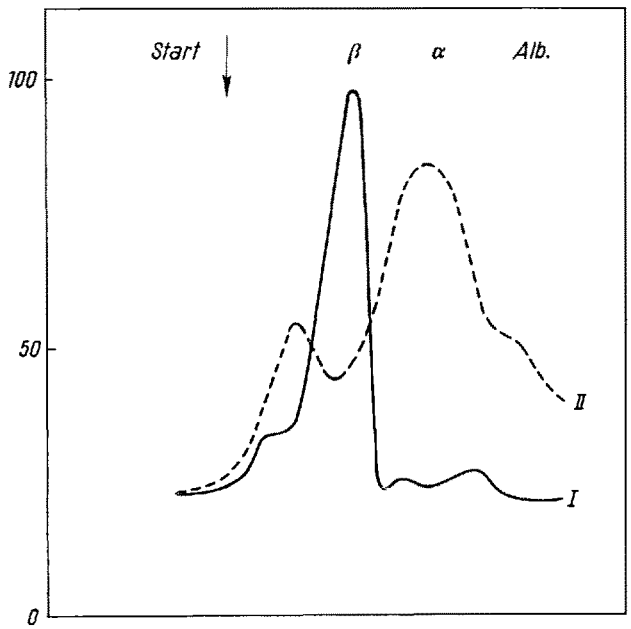
Note: Total cholesterol in globulins expressed in mg%. Ratio cholesterol in α-globulin/cholesterol in β-globulin showed a statistically significant increase in group 2 against group 1 at $P < 0{\cdot}02$; $t = 3{\cdot}866$. Group 3 was not analysed statistically owing to an insufficient number of observations.

Rabbits Nos. 1, 2 obtained Tween 80 (0·6%) and monoglyceride K 1 % in 5% glucose. Rabbits Nos. 3, 4 were given 5% glucose only.

according to ANDERSON and KEYS[2]. In atheromatous animals which received lipid emulsion infusions, pronounced changes in the lipoprotein spectrum were observed. The Figure shows that, prior to infusion, the major part of sudan stainable lipids are bound in the form of β-lipoproteins. After fat infusion the lipids shifted into the region of higher electrophoretic mobility with clear cut component consisting probably of chylomicra remaining near the start. No such effect was seen either after infusion of stabilisers or after glucose infusion. Cholesterol (Chol) analysis of the individual protein fractions prior to infusion revealed the major part of Chol to be in the β-globulin region whereas the Chol content in the α-globulin region was much smaller. The ratio Chol in α-globulin/Chol in β-globulin is thus smaller than one. No changes after infusion were observed in the control group. Lipid infusions however cause a basic change: the ratio Chol in α-globulin/Chol in β-globulin increased above one; it thus showed a tendency to normalise (Table). This was caused by a shift of a larger amount of Chol from β-globulin to the α-globulin region.

Observed changes are similar to those after heparin application[3]. It is therefore felt that these changes, similarly as in humans and dogs, can be caused by the lipolytic activity of the blood which is elicited upon lipid infusion[4,5]. After both types of lipid infusions, we have really found evidence of lipolysis in rabbit blood[6]. This was apparent as an increase in non-esterified fatty acids (UFA) in plasma. Previously we have found that blood changes observed after heparin injection, e.g. increase in UFA, occur primarily *in vitro*[7]; there are similar conditions after lipid infusions. It is worth noting that whereas phospholipid infusion causes a sharp increase of blood phospholipids *in vivo*, no such effects occur following neutral fat application. These facts are of some significance, since incubation of UFA[8] as well as of phospholipids[9] with serum causes the same lipoprotein changes as found after lipid infusions. The plasma postinfusion level of UFA *in vivo* is too small to cause lipoprotein changes. On the contrary, it is possible that the high concentration of blood phospholipids following lecithin infusion facilitates the lipoprotein changes reported to occur also in circulating blood, i.e. *in vivo*. This fact may perhaps contribute among other factors, to the explanation of the re-

Blood lipoprotein spectrum before and after infusion of neutral fat emulsion in atheromatous rabbits



Note: The bands stained with sudan black were photographed and the films photometered with the microphotometer MF-2. Blackening (in arbitrary units) was plotted against distance from the start. Curve I corresponds to lipid content in the protein fractions before lipid infusion, curve II after infusion.

Representative example.

[2] J. T. ANDERSON and A. KEYS, Clin. Chem. *2*, 145 (1956).
[3] E. NIKKILA, Scand. J. clin. Lab. Invest. *5*, Suppl. *8* (1953).
[4] F. S. M. HERBST, W. F. LEVER, and W. R. WADDEL, Science *123*, 843 (1956).
[5] W. F. LEVER and B. BASKYS, J. Invest. Dermat. *28*, 317 (1957).
[6] V. FELT and D. REICHL, unpublished results.
[7] V. FELT and D. REICHL, Čas. lék. čes. *96*, 954 (1957).
[8] R. S. GORDON, J. clin. Invest. *34*, 477 (1955).
[9] B. A. SACHS and E. DANIELSON, Proc. Soc. exp. Biol. Med., N. Y. *93*, 22 (1956).

trogression of atheromatosis in rabbits only after infusion of phospholipid emulsion.

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Zusammenfassung

Es wurde der Einfluss von Fettemulsioninfusionen auf das Serumlipoproteinspektrum von atherosklerotischen Kaninchen untersucht. Nach Lipoidinfusionen kommt es zu einer Verschiebung des Cholesterols und der sudanfärbbaren Lipoide aus dem Bereich der β-Globuline in jenen der α-Globuline.

A Consummatory Situation The Effect of Eggs on the Sexual Behaviour of the Male Three-Spined Stickleback (*Gasterosteus aculeatus* L.)

The decrease of a certain motivation after the performance of the appropriate consummatory act (CRAIG[1]) is a well-known phenomenon. There has been much discussion, however, as to how this phenomenon is effected. The original view (LORENZ[2]), that the performance of the consummatory act should actually consume motivation, has largely been abandoned. It has been pointed out that consummatory acts may effect a change in the external and/or internal situation, and it has been proposed that it is this new situation which stops the activity of the appropriate functional complex, rather than the performance as such of the consummatory act (see e.g. BASTOCK *et al.*[3]). In the male three-spined Stickleback fertilization is followed by an immediate decrease of sexual motivation, as measured by the number of zig-zags performed towards a female in a glass-tube ('sex-test', see VAN IERSEL[4]), and in this case the reduced pressure in the gonads, resulting from the decreased amount of sperm, has been proposed to be the 'consummatory situation' which stops sexual activity. Since in my present research on the subject of Stickleback behaviour the causation of this change in sexual behaviour has my special attention, I undertook to determine the exact influence of the act of fertilization experimentally. Two types (1 and 2) were designed such as to differ only in that fertilization did or did not occur.

(1) A male is given a sex-test of 1 min and the number of zig-zags is counted. The average of 10 experiments is 41·5/min. Then a female is introduced in the tank and is courted, until it creeps into the nest to deposit its eggs. When the female comes out the male creeps through the nest and fertilizes the clutch. Immediately the female is taken away, and so is the nest with the cluth. Another male's empty nest is given in return. A second sex-test of 1 min immediately after exchanging the nests (1–2 min after fertilization) yields an average of 4·6 zig-zags/min.

(2) In the second type of experiment the procedure is the same up to the moment when the female is depositing eggs. When it has almost finished with this, a ring of thin copperwire is put in the nest-entrance against the clutch, so that it prevents the male's entering the nest, as the diameter of the ring is about that of the male's head just before or behind the eyes. However, while trying to creep in for fertilizing it can and does amply touch the eggs. It is allowed to 'sniff' at the clutch in this way for 30 s, thus giving it at least as much opportunity to perceive the eggs as in the normal cases, in which the males may spend a similar time before fertilizing. When the ring has been put in position the female is removed as soon as it leaves the nest. After the 30 s period the nest with the eggs is replaced by an empty one. Of 13 experiments, the average number of zig-zags before the introduction of the female is 36·8/min; immediately after exchanging the nests it is 4·4/min.

To test the possible criticism that sperm might be ejaculated during the 30 s period of intention fertilization movements and 'sniffing' at the eggs, 16 clutches which had been subjected to such a period were cultured in glass-jars. My supposition, which was tested and verified by another experiment, was that if sperm were ejaculated it would be attracted by the eggs over the distance of about 2 cm from the male's genital pore to the nest-entrance. In 4 out of these 16 controls the males had been very persistently trying to get into the nest, and succeeded to get half-way through the ring. In 2 out of these 4 cases I found 4 developed eggs, in the remaining 14 controls none at all. So, during the 30 s period at the most very little sperm is ejaculated. Ejaculation of such an amount of sperm has no reducing influence on sexual behaviour: I found, by similar methods, that during 'creeping through' the nest (VAN IERSEL[4]), a movement very similar in form to the movement of fertilization, very often some sperm is ejaculated, and after 'creeping through' the number of zig-zags is higher than before (the average increase being 27%; P. SEVENSTER, personal communication).

From these results I must conclude that the act of fertilization is not the cause of the decrease of sexual motivation as described in this report. Nor can it be ascribed to the performance of the other sexual activities which precede fertilization, comprising zig-zagging, leading, showing the nest-entrance, and finally quivering on the female's tail when it is in the nest. (For description of these movements see TER PELKWIJK and TINBERGEN[5]). This fact is demonstrated a. o. by another of my experiments, in which the male was presented with a fresh clutch obtained by stripping and put into the nest by hand. So in this case no courting occurred (nor fertilization). The few (4) experiments of this type done thusfar yield an average of 40·7 zig-zags/min before, and of 2·5 zig-zags/min after egg presentation.

I then finally propose that the decrease of sexual motivation as described here is due to the male's *exposure to the eggs*, and that hereby an example is given of a real consummatory situation – which is *not* brought about by the performance of a consummatory act.

The author is indebted to Mr. P. SEVENSTER for cooperation in some of the control-experiments, and to Mrs. Dr. N. Z. ULMER, Dr. J. J. A. VAN IERSEL, and Mr. P. SEVENSTER for reading the manuscript.

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Zoological Laboratory of the Rijksuniversiteit, Leiden, December 23, 1958.

[1] W. CRAIG, Biol. Bull. 34 (1918).

[2] K. LORENZ, Naturwissenschaften *25*, 289, 307, 324 (1937).

[3] M. BASTOCK, D. MORRIS, and M. MOYNIHAN, Behaviour VI, *1*, 66 (1953).

[4] J. J. A. VAN IERSEL, Behaviour, Suppl. III, 119 (1953).

[5] J. J. TER PELKWIJK and N. TINBERGEN, Z. Tierpsych. *1*, 103 (1937).