THE BLOOD CHOLESTEROL FOLLOWING REPEATED ADMINISTRATIONS OF CHLOROFORM, PARALDEHYDE, AND URETHANE.

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During the course of some experiments necessitating the repeated administration of chloroform to rabbits, an increase in fat in the Kupffer cells and capillaries of the liver was noted. Since usually the cholesterol increases are more or less parallel to the increase of fat and lecithin (1), a quantitative determination of the former substance was made. The amount of cholesterol in the blood was found to be far above normal.

With this finding in view, a series of experiments was instituted to determine the constancy and period of appearance of this hypercholesterolemia. Paraldehyde and urethane were administered both as a control to the chloroform and also as a parallel study in the possible general relationship between narcosis and hypercholesterolemia. The only report in the literature that had any bearing upon this work was that of Duccheschi (2) who administered chloroform by inhalation to dogs for from 1 to 1½ hours daily for several days. He found that the serum cholesterol increased in moderate degree during the first 2 to 3 days of narcosis and then diminished rapidly until it was reduced to unusually low values. His dogs lived only a few days. Our results do not agree with those of Duccheschi but the methods were somewhat different. Our period of experimentation was longer, and the animal we used, the rabbit, was a different one than the one he used, the dog.

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Methods.—The chloroform was administered to the rabbits in one of two ways, either by injection subcutaneously or by inhalation. By the former method, 0.5 cc. of chloroform was injected into the subcutaneous tissue of the groin and after a lapse of 1 week 0.25 to 0.5 cc. of chloroform was given in a similar way every other day. The great mortality of the animals receiving chloroform subcutaneously made it advisable to do most of the experiments by the inhalation method, although one rabbit did receive 34.2 cc. subcutaneously in a little over 9 weeks.

The animals which were given inhalations were anesthetized for ½ hour daily for a period of 2 weeks and then 1 hour daily for 2 more weeks. After a month they were placed, for 1 hour per day, in a large covered box which contained cotton saturated with chloroform. The period in the box was increased rapidly until they were kept there for 4 hours. By this means the rabbits were constantly in a chloroform atmosphere, and although they were only lightly anesthetized, still, an overdose of chloroform, to which rabbits are highly susceptible, was prevented. The box method has the important advantage of being time-saving.

Paraldehyde and urethane were given by stomach tube; 1 gm. per kilo being the dose used as advised by Sollmann (3).

The cholesterol was determined by the method described by Leiboff (4). Standards of different strengths were used and the unknown was read against the nearest standard. In every instance when a cholesterol determination was made upon the blood of an experimental animal, it was also made at the same time and under the same conditions upon the blood of one or more normal rabbits; thus the chemical method was constantly checked. The blood was usually withdrawn in the morning, before the next dose of chloroform was administered. The repeated withdrawal of blood did not affect the cholesterol. This was found experimentally. The quantity used was probably too small to cause any change.

Blood Cholesterol in Normal Rabbits.

The serum blood cholesterol in normal rabbits has been determined by Grigaut and coworkers (5) to average about 40 mg. per 100 cc. Weidman and Suderman (6) found it to vary between 64 and 80 mg. For whole blood Clarkson and Newburgh

(7) estimate 71.3 mg. per 100 cc. as the average, while Harnes (8) gives the range for his rabbits as from 51.1 to 73.3 mg. per cent. In our rabbits, both those used in these experiments and in many controls, the readings in blood serum cholesterol determinations upon 66 animals, ran between 19 and 100 mg. per 100 cc. with an average of 46.6 mg. per 100 cc.; most of the figures, however, were between 30 mg. and 80 mg. per 100 cc. Four results, namely 15, 19, 23, and 24 mg. per 100 cc. were below 30 mg. per 100 cc.; four—82, 83, 93, and 100 mg. were above 80 mg. per 100 cc. The wide variation, due to the yet imperfect methods available and to the greater difficulty that obtains in the rabbit whose blood cholesterol is low, was also experienced by Clarkson and Newburgh (7) and Harnes (8). In whole blood the former found the cholesterol of normal rabbits to vary between 35 and 125 mg. per cent, while the uncorrected figures of the latter run from 30 to 99.9 mg. per cent. It is evident then that only pronounced changes in the blood cholesterol can be considered of any interpretive value.

Blood Cholesterol in Chloroform Administration.

I. Subcutaneous Administrations.—Four animals were used in this series, three of whom had been given chloroform before blood cholesterol determinations were made. The fourth animal was studied rather carefully. See Table I.

The blood cholesterol values in the other rabbits were:

Rabbit 550; 240, 182, 232, and 193 mg.	Chloroform	administered	9 wks.
" 134; 137 mg.	"	"	19 days.
" 112; 119 "	"	"	1 mo.

II. Administration by Inhalation.—Eleven animals comprise this series. A typical protocol is given in Table II.

The blood serum determinations of the other rabbits gave the results shown in Table III.

Blood Cholesterol in Urethane Administration.[1]

Table IV indicates that urethane does not produce the rise in cholesterol that is found after repeated chloroform administration.

[1] Both urethane and paraldehyde were given every 5 to 7 days.

Except for one figure, 173 mg., the cholesterol readings are all practically within the range of the normal controls although it is

TABLE I.

*Cholesterol Determinations for Rabbit 593.**

Date.	Cholesterol.	Date.	Cholesterol.
	mg. per 100 cc.		*mg. per 100 cc.*
Apr. 21	74	May 23	220
May 8	73	" 26	175
" 15	110	June 3	140
" 19	150	July 7	156
" 21	190		

* On April 22, 0.5 cc. of chloroform was given subcutaneously, and commencing May 8, 0.5 cc. of chloroform was given every 2 days.

TABLE II.

Rabbit 473.

Date.	Length of exposure to chloroform.	Cholesterol determination.	Date.	Length of exposure to chloroform.	Cholesterol determination.
	hrs.	*mg. per 100 cc.*		*hrs.*	*mg. per 100 cc.*
Mar. 18	½	40	Apr. 28	2	100
" 19	½	11	May 5	4	230
" 25	½	68	" 13	4	100
" 31	½	42	" 24	4	170
Apr. 11	1	73	" 26	4	145

TABLE III.

Blood Serum Determinations on Rabbits.

Rabbit No.	Normal cholesterol.	Cholesterol determination.	Rabbit No.	Normal cholesterol.	Cholesterol determination.
	mg. per 100 cc.	*mg. per 100 cc.*		*mg. per 100 cc.*	*mg. per 100 cc.*
79	33	147 after 5 mos.	713	34	114 after 1 mo.
475	43	180 " 10 wks.	187	81	163 " 2 mos.
966	74	120 " 4 mos.	189		121 " 2 "
172	46	133 " 2 "	51		166 " 10 wks.
188	78	163 " 1 mo.	53		179 " 11 "

interesting to note that in this series the average of the normal readings gives 48.4 mg. whereas the average of the readings in the

animals who had received more than two doses of urethane is 85 mg. per 100 cc. This might possibly be interpreted as a slight rise.

Blood Cholesterol in Paraldehyde Administration.

In these experiments the number of animals are fewer and the results are less definite. (See Table V.)

TABLE IV.

Blood Cholesterol after Urethane Administration.

Rabbit No.	Normal cholesterol.	Cholesterol determination.
	mg. per 100 cc.	*mg. per 100 cc.*
397	Too low.	94 after 7 doses, 56 after 16 doses.
603	30.3	85 " 6 "
601	36.3	98 " 6 " 173 after 14 doses.
602	69.4	96 " 7 "
395	68.9	59 " 7 "
934		80 " 2 "
645	37	73 " 6 " 54 after 14 doses.
936		54 " 2 "
710		71 " 5 " 62 after 13 doses.
711		102 " 5 "

TABLE V.

Blood Cholesterol after Paraldehyde Administration.

Rabbit No.	Normal cholesterol.	Cholesterol determination.
	mg. per 100 cc.	*mg. per 100 cc.*
735	24	79 after 6 doses.
734	83	56 " 4 "
103	82	68 " 4 "
938		74 " 3 "
742		40 " 2 " 105 after 8 doses.
743		40 " 2 " 40 " 8 "
939		56 " 2 "
937		62 " 2 "

On the whole the indications are that repeated administration of paraldehyde does not produce an increase in blood cholesterol.

SUMMARY AND CONCLUSIONS.

There appears to be a definite rise in blood cholesterol beginning about 3 weeks after repeated administration of chloroform. If

the chloroform administration is continued for a longer period, the cholesterol level remains high, but does not show a progressive rise. No very marked change in the blood cholesterol occurs after repeated administration of urethane or paraldehyde, although we cannot, at the present time, rule out the possibility that a very slight increase in cholesterol takes place after repeated urethane administrations.

As to the significance of the increase in cholesterol in the blood under those conditions no definite statement can be made. Further investigations are being carried out to determine this.

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