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DEPOSITION OF FAT IN CORONARY ARTERIES AFTER EXPOSURE TO COLD*

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[WITH SPECIAL PLATE]

Very few physiological or nutritional investigations have been made on the effects of prolonged exposure to a constant cold environment. Growing interest in the application of hypothermia to surgery, in injuries produced by cold, and in changes associated with acclimatization to low temperatures has prompted a series of studies in our laboratory. An incidental observation, which may have some clinical significance in the future, concerns the presence of fatty deposits in the coronary arteries of the rat after prolonged exposure to cold.

When rats are exposed to a cold environment (1° – 3° C.) for long periods many metabolic changes take place. Increased heat production is necessary to maintain body temperature, and this higher expenditure of energy results in a large increase in oxygen consumption and in food intake. Changes in carbohydrate, fat, and nitrogen metabolism and in adrenal, thyroid, and pituitary function are observed. No doubt most of these changes are adaptive phenomena associated with the augmented ability of the rat to survive under adverse environmental conditions. Over a long period of time, however, it might be anticipated that adverse effects on certain tissues might occur. In spite of the fact that after 12–24 months of exposure to cold the gross appearance of the visceral organs of the rat is not much altered, the weights of many of the viscera are increased and histological evidence of structural changes is present. Deposition of fat in the intima and media of the coronary arteries was observed by one of us several years ago and referred to in discussion (Sellers, 1955). In the present paper the details of this finding will be reported. An experiment will also be described in which diets of high fat, high cholesterol content, with and without a choline supplement, were fed to rats at room temperature and in the cold.

Because of the association of this type of arterial lesion with lipid, and especially with cholesterol, metabolism, in the first experiment the total lipids and serum cholesterol were estimated in the blood of rats exposed to cold for long periods.

Experimental Investigation

Experiment 1

Male and female Wistar rats weighing 120–220 g., bred in the laboratory, were placed in individual metal cages in

a cold room kept at 1° – 3° C. They were offered the stock ration (Fox breeder cubes, Master Feeds, Toronto Elevators Ltd.) and water *ad libitum*. After 10–18 months they were killed either with ether vapour or by amylobarbitone sodium injection. The heart, aorta, kidneys, and sometimes other tissues were removed, weighed, and fixed. Bouin's solution was used for routine fixation, although formalin was used as fixative before staining for fat with oil red O (Wilson technique). For the histological study 29 animals which had been exposed to cold and 27 control animals of comparable age were used. Blood pressure was measured by inserting a plastic cannula directly into the aorta while the animals were under light ether anaesthesia, readings being obtained from 44 rats which had been exposed to cold for 10–18 months. With one group of 9 animals blood pressure was taken indirectly (Friedman and Freed, 1949) without anaesthesia, and compared with measurements of 10 controls of the same age kept at room temperature. Whenever blood pressure was to be measured the animals were removed from the cold room approximately half an hour preceding the estimation.

Experiment 2

Blood lipids were estimated in rats of both sexes (225–395 g.) which had been exposed to an environment of 1° – 3° C. for 1–5 months under the conditions mentioned above. Food containers were removed from the cages 1–4 hours before the animals were killed. The rats were anaesthetized by intraperitoneal injection of amylobarbitone sodium, and blood was collected in conical centrifuge tubes from the severed aortas. The blood was allowed to clot at room temperature and then kept overnight under refrigeration. After separation the serum obtained from 2–7 rats was pooled and the lipid estimated on duplicate samples essentially as outlined by Bloor *et al.* (1922) and modified by Boyd (1933). The final values for total lipid were obtained by weighing the dry residues after rectification with petroleum ether–chloroform. Cholesterol was precipitated as digitonide and determined colorimetrically as described by Sperry and Webb (1950). The results were compared with control values obtained with similar techniques from animals of similar age and sex, kept at room temperature ($22 \pm 2^{\circ}$ C.). The blood lipids and serum cholesterol of a third group of male rats which had been in the cold for 12–13 months were estimated similarly. In the case of this latter group the sera of 2 rats only were pooled. In all, some 150 rats were used in the experiment.

Cholesterol estimations were carried out on the individual sera of an additional 6 male rats which had been exposed to cold for 9 months, and on 6 control animals from the rat colony (about 25° C.). Unfortunately the controls were somewhat lighter in weight (average 200 g.) and younger than the "cold" animals (average weight 290 g.).

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Experiment 3

Sixty male rats, 149–155 g., were divided into four groups of 15. Two groups were fed a high-fat, hypolipotropic diet containing 2% cholesterol (alcohol-extracted peanut meal 28, casein 7, washed alpha soya protein (Glidden Co., Chicago) 5, salt mixture 3, vitamin mixture 1, sucrose 11, starch 8, lard 34, cholesterol 2, cod-liver-oil concentrate 0.015, α -tocopherol acetate 0.01; the composition of the salt mixture and of the vitamin mixture was that used by Best *et al.* (1953)). The rats were kept in individual cages, one group in the rat colony ($22 \pm 2^\circ \text{C}$), the other in a cold room at $1^\circ\text{--}3^\circ \text{C}$. The two remaining groups were fed a similar diet supplemented with 0.5% choline chloride, and were kept one at $22 \pm 2^\circ \text{C}$, one at $1^\circ\text{--}3^\circ \text{C}$. All animals were fed *ad libitum*. After six weeks the surviving animals were killed, the organs weighed, and the tissues prepared for histological examination. The livers, which were remarkably consistent in appearance within groups, were pooled and total liver lipids estimated (Best *et al.*, 1946). Tissues of animals dying during the experiment were also fixed in Bouin's solution and in formalin for subsequent histological study.

Results

Experiment 1

Vascular Lesions.—Of 29 animals which had been exposed to cold for 10–18 months, 18 had stainable lipid in the coronary arteries (Special Plate, Figs. 1 and 2). No fat could be found in any of the coronary vessels of the 27 control rats. In both groups, one section was cut at random transversely from the mid-portion of the heart. Fat occurred in both the intima and media. The intimal cells and sub-endothelial tissues were swollen and contained diffuse, red-staining material which was not bi-refrinent under polarized light. In some cases proliferating intimal cells, phagocytes laden with lipid material, and degenerating tissue encroached on the lumen. In the media the muscle cells were swollen and contained fat stained pink or, occasionally, brilliant red. These two lesions did not always occur together, so that a temporal relationship of one to the other cannot be inferred. Arteries of moderate size appear to be involved more than either large or small vessels. In longitudinal sections it was seen that often only part of an artery was affected. This probably accounts for the absence of visible lesions in many random transverse cuts of vessels. In many sections of the aortas there was a reduplication of endothelial cells, as seen by an increase in number of nuclei and occasional “layering” of cells. In three or four the swollen endothelial cells were sudanophilic. In a similar number (not necessarily the same sections) sudanophilia was present in the media. These changes were not seen in any of the aortas from control

rats. No fat was demonstrated in the arteries of the kidney in experimental or control animals. In both controls and in “cold” animals, arteritis, or periarteritis, especially in vessels of the lungs, was relatively common. Occasionally the coronary vessels were involved. This same finding has been described by others (Wilens and Sproul, 1938; Wissler *et al.*, 1954).

Myocardial Lesions.—Small areas of sudanophilic “stippling” were occasionally seen in sections stained for fat.

Blood Pressure.—Blood pressure was measured directly in 44 “cold” rats under light ether anaesthesia; all values were within the normal range (102 ± 2 mm. Hg). By the indirect method 3 of 9 “cold” rats had values of 165–175 mm. Hg, although the average for the group (143 ± 8 mm.) was not significantly higher than that (130 ± 5 mm.) of the control group of 10 rats kept at room temperature.

Organ Weights.—The hearts and kidneys of 17 rats kept in the cold were significantly heavier than those of 10 controls kept at room temperature (hearts: 0.53 ± 0.03 g. per 100 g. cold; 0.31 ± 0.004 g. per 100 g. room temperature; kidneys: 0.96 ± 0.04 g. per 100 g. cold; 0.62 ± 0.02 g. per 100 g. room temperature). The adrenals were usually two to three times the weight of those of controls, for both males and females.

Kidneys.—Pathological changes in the kidneys varied greatly from animal to animal, the lesions being most advanced in rats exposed to cold for the longest periods. The findings consisted of: (1) degeneration of tubular cells with deposition of fat in the cells; (2) dilatation of tubules with appearance of casts which often were fatty; (3) appearance of fat in cells of the glomerular capsule or in the basement membrane adjacent to glomerular capillaries; (4) ischaemia and deposition of hyaline material in loops of the glomerular capillaries; and (5) no major changes or deposition of fat in renal arterioles or arteries.

Experiment 2

The results of the second study are shown in Tables I and II. The concentration of blood lipids of rats exposed to cold for 12–13 months is significantly higher than that of controls or of rats exposed to cold for 1–5 months. The concentrations of free and bound serum cholesterol are also higher in animals exposed to cold for more than 9 months.

Experiment 3

The results of Experiment 3 are given in Table III. Intake of food in the cold was considerably greater than at room temperature, but the growth rate (increase in weight) was less. The content of lipid was extremely high in the livers of rats fed the choline-deficient diet at room temperature;

TABLE I.—Effect of Cold on Total Lipids and Cholesterol Concentration (in mg. per 100 ml.) in Serum of Rats

Group	Sex and No.	Controls ($22 \pm 2^\circ \text{C}$)			Cold Exposure ($1^\circ\text{--}3^\circ \text{C}$) 1–5 Months				Cold Exposure ($1^\circ\text{--}3^\circ \text{C}$) 12–13 Months			
		Total Lipids	Cholesterol		Sex and No.	Total Lipids	Cholesterol		Sex and No.	Total Lipids	Cholesterol	
			Bound	Free			Bound	Free			Bound	Free
1	F (4)	455	58.1	24.4	M (2)	604	53.3	13.0	M (3)	1,056	—	—
2	F (6)	533	58.1	20.7	M (4)	353	45.3	12.9	M (2)	776	—	—
3	F (4)	523	48.1	15.7	M (5)	342	44.2	12.7	M (2)	624	71.8	20.8
4	M (4)	382	35.3	9.7	M (4)	487	44.6	16.7	M (2)	622	72.8	19.8
5	M (3)	404	40.5	12.0	M (5)	362	50.3	15.4	M (1)	632	62.2	20.4
6	M (6)	264	34.2	10.9	M (4)	290	38.1	11.9	M (2)	764	83.2	28.0
7	F (7)	356	41.7	15.2	M (4)	326	49.5	14.9	M (3)	494	38.6	9.0
8	F (5)	431	43.2	15.6	M (5)	338	47.5	13.8	M (3)	930	97.6	27.4
9	M (2)	534	—	—	F (6)	360	47.7	16.1	M (2)	724	64.0	21.0
10	M (2)	310	—	—	F (5)	399	50.8	16.7	M (2)	874	83.0	25.8
11	M (2)	382	—	—	F (2)	468	57.3	17.7	M (2)	394	63.6	16.4
12	M (3)	410	—	—	M (2)	528	—	—	M (2)	316	67.8	13.4
13	M (2)	434	37.2	10.4	M (3)	536	—	—	M (2)	570	76.2	26.4
14	M (2)	530	46.0	12.8	M (4)	576	—	0	M (2)	600	41.8	29.4
15	—	—	—	—	—	—	—	—	M (2)	754	—	—
Avg.		424.9	44.2	14.7		426.4	48.1	14.71		675.3	68.6	21.5
S.E.		± 22.7	± 2.7	± 1.5		± 27.7	± 1.5	± 0.6		± 50.8	± 4.8	± 1.81
P=		—	—	—		—	> 0.10	—		< 0.01	< 0.01	$0.02\text{--}0.01$

TABLE II.—*Effect of Cold Environment on Serum Cholesterol (Rats). Total Cholesterol, mg. per 100 ml. Serum*

Rat No.	Cold (1°-3° C., 9 Months)	Control (22±2° C.)
1	82.0	50.0
2	50.0	59.0
3	66.6	46.5
4	47.7	50.0
5	83.4	45.3
6	75.0	46.5
Av.	67.5	49.6
S.E.	±6.37	±2.1
P=		<0.05 >0.02

TABLE III.—*Some Effects of Diets High in Fat, with 2% Cholesterol*

	Cold Environment (1°-3° C.)		Normal Environment (22±2° C.)	
	1 Choline-deficient	2 Choline 0.5%	3 Choline-deficient	4 Choline 0.5%
No. of rats	15	15	15	15
No. of survivors	8	11	5	15
Body wt. (g.), initial	153.0	149.0	155.0	151.0
" " final	241.0	252.0	243.0	305.0
Food consumption (g. per day)	17.0	17.6	11.5	14.5
Liver wt. (g.)*	20.8	12.7	23.6	11.9
" total lipids (wet wt.) (%)	33.5	12.7	38.2	16.7
Kidneys (g.)*	2.5	2.7	3.0	2.2
Adrenals (mg.)*	47.9	51.5	47.0	45.6
Heart (g.)*	1.2	1.7	0.9	1.0

* Averages of survivors only.

it was somewhat less in the group receiving this diet in the cold. The addition of choline to the diet greatly reduced the level of hepatic lipids at room temperature and in the cold. Two-thirds of the group receiving the choline-deficient diet at room temperature died before the end of the experiment. At necropsy severe "haemorrhagic" kidneys (Griffith and Wade, 1939) were observed. Almost half the group receiving this diet in the cold failed to survive. The kidneys of "cold" rats were less affected, in some cases being almost normal in appearance. This suggests that the exposure rather than damage to the kidneys *per se* was an important contributory cause of death. Animals which survived the experimental period showed the following changes:

Room-temperature Group—With Choline.—The kidneys were normal in macroscopic appearance. Histologically, a few small deposits of lipid were seen in glomerular cells. The livers were paler than normal, and histologically the parenchymal cells contained fine droplets of stainable lipid distributed around portal areas (cholesterol fatty liver). The hearts appeared normal to the naked eye and, with one exception, histologically. In this one rat lipid was present in the subendothelium and media of a medium-sized coronary artery (Plate, Fig. 3).

Room-temperature Group—Without Choline.—The kidneys were enlarged, dark red, and microscopically showed the extensive tubular and haemorrhagic lesions associated with haemorrhagic kidneys (Griffith and Wade, 1939). The livers were large and yellow in colour, and histologically the parenchymal cells were seen to contain large amounts of sudanophilic material. The heart showed no gross changes. Microscopically a few fibres were stippled with sudanophilic material.

In hearts from rats which failed to survive (5 examined) at least one vessel, and usually several vessels from each rat, had lipomatous deposits in the intima and media (Plate, Fig. 4). The kidneys were severely damaged.

Cold Group—With Choline.—The kidneys were essentially normal. The livers were paler than usual, and fat stains revealed fine fat droplets in parenchymal cells distributed around portal areas. The amount was less than in

controls kept at room temperature. In 50% of the animals the coronary vessels had deposits of lipid in the intima and media. The appearance varied somewhat, but in most cases the lipid appeared in droplets rather than diffusely. Partial blocking of the lumen by lipophages, proliferation of intimal cells, and degenerating tissue were observed in some, but not all, affected vessels (Plate, Fig. 5). Many of the vessels (small arteries, sinusoids, veins) were partially filled with a granular material which appeared to be adherent to the endothelial lining of the vessels. This material stained pink or red with oil red O, and in the microsection frequently separated red cells in the vessel from the endothelium. Its appearance, although undoubtedly affected by the preparation of the tissue for histological study, suggested a physical barrier between blood cells and vessel cells (Plate, Figs. 6 and 7). Many of the endothelial cells subjacent to such masses were swollen and contained fat. The muscle fibres were less affected in this group than in the group in the cold not receiving choline, but showed "sudanophilic stippling" in scattered areas. In paraffin sections loss of striations, vacuolation, and hyalinization together with nuclear degeneration occurred in corresponding locations.

Cold Group—Without Choline.—The extent of damage to the kidney was less than in the control group kept at room temperature, some kidneys being almost normal. The livers were yellowish, and microscopically were seen to contain fat in large globules within parenchymal cells and in "fatty cysts." The size and number of the globules were smaller than in the control group at room temperature. The heart muscle of most rats in this group was stippled with sudanophilic material; in a few animals large areas were involved (Plate, Fig. 8), but in most only a few scattered fibres were affected. In sections stained with haematoxylin and eosin (Fig. 9a and b) the most severely affected parts were seen to be undergoing necrosis. In spite of these widespread lesions of the myocardium, no arteries or arterioles were found which contained stainable lipid in their walls.

Discussion

So far as we know this is the first report of cardiovascular lesions being produced in normal animals, fed a normal diet, by changing only the environmental conditions. The animals used as controls were of similar strain, sex, and age, but otherwise were not selected. Because of limitations of space the control animals were not always kept in individual cages. To what extent this difference in living conditions might affect the outcome of such a study is unknown. We have assumed that the influence of companionship on coronary arteries is negligible. No lipomatous lesions of the coronary vessels were seen in 20 or 30 rats fed a diet containing 10% fat for an approximately similar period of time. Deposition of fat in coronary vessels in rats of our colony fed the stock diet has been a rare occurrence indeed within the age limits concerned.

A number of possible explanations may be advanced for the abnormal deposition of fat in the coronary vessels.

Premature Ageing.—Spontaneous lesions of the coronary vessels and of the aortas have been described by Hummel and Barnes (1938) and by Wilens and Sproul (1938) in rats more than 600 days old. As stated above, in our own colony such lesions have been of rare occurrence, even in rats examined after attaining an age of 750 days (Hartroft *et al.*, 1952). Nevertheless in many species fat deposition in, and degenerative changes of, the arterial system are associated with the ageing process. Increased metabolic activity involving skeletal muscle (Sellers and Kalant, unpublished) and many visceral tissues has been demonstrated in rats exposed to cold (You and Sellers, 1951). When such activity is maintained for many months it might be expected to hasten the process of growing old.

Hormonal Factors.—The effect of hormones, especially those of the adrenal gland, on vascular lesions in the heart,

aorta, and kidneys has been reported by numerous workers. For instance, Selye *et al.* (1943) have produced nephrosclerosis with hypertension by administering deoxycortone acetate to rats, especially if these were unilaterally nephrectomized and given saline to drink instead of water. In rats exposed to cold the adrenals become larger than those of controls kept at room temperature, and remain large for the duration of exposure (as long as two years). The oxygen consumption and succinic dehydrogenase activity of adrenal tissue measured *in vitro* are both increased (Sellers and Kalant, unpublished).

Kidney.—Lesions of the kidney occur in rats kept a few degrees above freezing for long periods (MacKay *et al.*, 1934). The incidence and severity of these changes vary with the duration of exposure to cold. The pathogenesis of the changes described is not clear. Selye (1943) and Leblond and Dugal (1943) have related the changes to the increased size and activity of the adrenal cortex, and have noted that nephrosclerosis with hypertension can be produced by exposing "sensitized" rats (unilateral nephrectomy, saline in lieu of water to drink) to cold for extended periods. The association of pathological changes in the kidney with hypertension and with vascular lesions in other parts of the body is well known.

Blood Pressure.—Several authors (Gilson, 1950; Thérien *et al.*, 1949; Fregly, 1954) have observed that the systolic blood pressure of the rat becomes elevated during prolonged exposure to cold. This is a reversible phenomenon, at least for a considerable period (16 weeks), since it does not persist when the animals are returned to room temperature (Gilson, 1950). Most of the measurements of blood pressure reported in this paper, unlike those of the previous workers, were taken directly under light anaesthesia with ether, and no hypertensive values were observed. Among the small group (9) in which indirect measurements were made several animals were apparently hypertensive. These findings suggest that the hypertension is a phenomenon in which nervous or hormonal factors might play an important part. Nevertheless, the persistence of a moderate hypertension (albeit reversible) while the animals are exposed to cold might be related to the renal and cardiovascular lesions.

Blood Lipids.—Evidence that the level of total lipids in the blood is elevated after prolonged exposure to cold is contained in Table I. The free and bound cholesterol were moderately but significantly elevated above control values in both groups studied after nine months' exposure. This fact, together with the results of the experiment in which a diet of high cholesterol content was fed to rats in the cold for six weeks, suggests that dietary lipid, especially cholesterol, may play a prominent part in the occurrence of lipoidosis of coronary vessels after exposure to cold. In this latter experiment, rats fed a choline-deficient diet in the cold did not have coronary lesions, although the myocardium showed "stippling" with sudanophilic material and other degenerative changes. It may be pertinent to recall here that Ridout *et al.* (1954) have shown that, in the absence of dietary choline, serum cholesterol values fall in spite of considerable cholesterol in the diet.

The work of Wilgram *et al.* (1954a, b) and the earlier report by Hartroft *et al.* (1952), both from this laboratory, have shown quite clearly that a deficiency of choline and other lipotropic factors results in vascular lesions similar in many respects to those described here. Evidence of extensive damage to the kidneys exists in animals showing vascular lipoidosis after receiving diets low in choline (Wilgram *et al.*, 1954a, b). In the present experiments there was no deficiency of dietary choline in the high-fat, high-cholesterol diet that was fed to the rats in which most, if not all, of the coronary lipoidosis was observed. In addition, exposure to cold in itself exerts a "pseudolipotropic" effect (Sellers and You, 1952) in so far as deposition of fat in the liver and production of haemorrhagic kidneys can be taken as indices of such an effect. The kidneys of these rats were essentially normal.

At room temperature coronary lipoidosis occurred without choline, and in the cold it occurred with choline; a different pathogenesis must exist in the two cases. It is interesting to confirm, in one experiment (performed under slightly different conditions), the apparently conflicting results of both Wissler *et al.* (1954) and Wilgram *et al.* (1954a, b). It is possible that the coronary and aortic lesions seen in animals exposed to cold for long periods may be related in some way to the pathological changes in the kidneys; it is also possible that they may be related to the level of dietary lipid. The blood lipids, especially cholesterol, were significantly increased in rats exposed to cold for lengthy periods. The commercial ration fed to these animals had an average fat content of only 3.2% and contained no fat of animal origin. Thus a change in the intermediary metabolism of fat during prolonged exposure to cold must be envisaged. The "pseudolipotropic" effect of cold referred to above would support this contention. Another finding of interest was the occurrence of myocardial degeneration in animals fed a high-cholesterol diet in the cold. It has been pointed out that the occurrence of myocardial damage (see also Wilgram *et al.*, 1954a, b) did not appear to be directly related to the arterial lesions, for myocardial degeneration was seen in rats receiving diets with and without choline, while arterial lesions were noted only in the group kept in the cold and receiving a high-cholesterol diet with choline. Whether the characteristics of the plasma lipid are altered by feeding a high-cholesterol diet to animals exposed to cold in such a way as to interfere with the oxygenation or nutrition of the myocardium from sinusoids and capillaries cannot be stated. The appearance of stainable fat adherent to and within the endothelial cells of these small vessels in sections stained for fat suggests such a possibility (Figs. 4 and 5).

The relationship between the frequency of the arterial and myocardial lesions and the time of their appearance with various levels and types of dietary lipids has not yet been studied under cold environmental conditions. The finding that coronary lesions may be produced in the rat fed a stock ration and exposed to cold for long periods indicates that further experimentation along these lines may be profitable. The production of similar lesions by feeding a diet containing 2% cholesterol and exposure to cold for a much shorter time suggests the direction this study will take.

Comment

Wistar rats fed a stock ration were kept in an environment of 1°–3° C. for 10–18 months. More than half developed a lipoidosis of the coronary vessels. In a few cases minimal lesions were present in the aortas also. Renal damage, varying in severity, was almost always present. The systolic blood pressure measured directly under ether anaesthesia was not elevated, although when an indirect technique was used a few animals appeared to be hypertensive.

Serum total lipids and free and bound cholesterol were significantly increased in rats fed the stock diet in a cold environment for a year or more.

Coronary lipoidosis was produced in rats fed a high-fat, high-cholesterol diet with choline and exposed to cold for six weeks. Myocardial lesions were also observed in such "cold" rats whether choline was present or not. Arterial lesions were not observed in "cold" rats fed the same diet without choline.

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LONGEVITY AND THE EARLY HISTORY OF THE TETRALOGY OF FALLOT

BY

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The history of the tetralogy of Fallot is long and fascinating; it includes some of the most famous names in medicine, and yet its own designation perpetuates the memory of a physician who has little other claim to fame. In spite of the complex nature of the malformation, in which there are pulmonary stenosis, a defect of the ventricular septum, dextroposition of the origin of the aorta, and right ventricular hypertrophy, it is the commonest form of cyanotic congenital heart disease in which survival beyond infancy is frequent.

In this paper are reported two patients who survived for an unusually long time and in whom the diagnosis was probably made in childhood by the late Dr. G. A. Gibson, well known for his contributions to the clinical recognition of patency of the ductus arteriosus (Gibson, 1900). The main references available to Dr. Gibson at the time he saw these patients are reviewed to illustrate the early history of the malformation.

Case Reports

Case 1.—Woman aged 64 years. Normal delivery at home in 1886. She was a "blue baby," but developed normally and was able to attend an ordinary school. She made good progress, but was always cyanosed even at rest and breathless on slight exertion. It was on this account that she was examined by Dr. Gibson in 1895. In her teens she could walk up to a mile without distress,

and when her parents died she was able to manage her own house, organize her servants, and go on annual holidays. As an only child of well-to-do parents she was able to lead a sheltered life without the need to earn her own living. During the last 18 months of her life she took little exercise. She did not rise until after lunch, and thereafter never went out for more than a quarter-of-an-hour walk. Three weeks before death she caught a cold and began to have attacks of dizziness and transient loss of memory. During her last week she became lifeless, mentally confused, and more deeply cyanosed. She was admitted to hospital for the first time in her life 24 hours before death in anoxic coma in 1951.

She was an obese, comatose woman with marked cyanosis, clubbing of the fingers, and polycythaemia. Heart rate regular at 100 per minute, blood pressure 150/104 mm. Hg, Hb 130% (Sahli). There was no clinical cardiac enlargement or evidence of heart failure. She had a loud pulmonary systolic murmur without accompanying thrill, and her pulmonary second sound was single and of diminished intensity.

Post mortem the clinical diagnosis of Fallot's tetralogy was confirmed. There were a severe grade of valvular pulmonary stenosis, a narrow infundibulum of the right ventricle, a large defect of the membranous portion of the interventricular septum, dextroposition of the aorta which overrode the ventricular septal defect, and a grossly hypertrophied right ventricle (Special Plate, Fig. 1). The atrial septum was normal, with valvular closure of the foramen ovale (Hudson, 1954). There was extensive infarction of the lower lobe of the right lung with thrombosis of many of the smaller vessels in that lobe. The beginning of the main pulmonary artery was dilated and its wall thin; the thickness of the right ventricular wall varied between 1 and 1.5 cm. The pulmonary valve cusps were fused to form a cone with a central orifice 4 mm. in diameter. There was a small calcified nodule adjacent to the anterior margin of the orifice on its pulmonary-artery side. The ductus arteriosus was closed. Microscopical examination of the lungs confirmed the recent thrombosis of the medium-sized pulmonary vessels with infarction of the right lower lobe. Throughout both lungs numerous small pulmonary vessels were abnormal. There was sinusoidal grouping of vessels so that two to four channels, separated by fairly narrow septa of collagenous tissue and lined by endothelium, were enclosed in what was apparently a single adventitial sheath (Plate, Fig. 2). The appearances are similar to those described by Rich (1948).

Case 2.—Man aged 47 years, born in 1905. He had been cyanosed at rest and breathless on exertion for as long as he could remember. When 4 years old he had been admitted to the Royal Infirmary, Edinburgh, under the care of Dr. Gibson for investigation of these symptoms. At

FIG. A.—Case 2: Electrocardiogram of patient at 46 years of age. There is striking evidence of right ventricular hypertrophy. A year later the T waves were no longer inverted in V1-V5.

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FIG. 1.—Coronary artery of medium size from rat kept at 1° – 3° C. for about a year. Endothelial cells swollen, increased in number, and contain fat (black in photomicrograph). Lumen partly occluded. In lower part fat can be seen in media. (Oil red O, Wilson. $\times 400$.)

FIG. 2.—Coronary artery of medium size from rat kept in cold for 10 months. Note extensive deposit of fat (black) in media and intima. Much contained in lipophages. (Oil red O, Wilson. $\times 400$.)

FIG. 3.—Coronary artery. Control rat ($22 \pm 2^{\circ}$ C.) fed high-fat, high-cholesterol diet with 0.5% choline chloride for six weeks. Endothelial cells slightly swollen; contain fine droplets of fat (black). Lipid present in media (lower left). Note similar changes in smaller vessel (upper left). (Oil red O. $\times 715$.)

FIG. 4.—Coronary arteriole. Rat died after three weeks; room temperature ($22 \pm 2^{\circ}$ C.); high-fat, 2% cholesterol diet without choline. Intima and media filled with lipid material (black). Note phagocytes in adventitia (upper left). (Oil red O. $\times 1070$.)

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FIG. 5.—Coronary artery. Rat given 2% cholesterol and 0.5% choline chloride in diet for six weeks and kept at 1°–3° C. Swollen endothelial cells filled with fat (black). Lipophages present. In media many muscle cells contain lipid. (Oil red O. Wilson. $\times 700$.)

FIG. 6.—Coronary sinusoid. Rat given 2% cholesterol and 0.5% choline chloride in diet for six weeks (1°–3° C.). Contains red blood cells, and granular sudanophilic material adherent to and within endothelial cells. (Oil red O. $\times 375$.)

FIG. 7.—Longitudinal section of small coronary vessel of rat receiving same diet in cold for six weeks. Note large droplets of fat (black) within walls, mostly in endothelial cells and lipophages. (Oil red O. $\times 375$.)

FIG. 8.—Myocardium from rat in cold (1°–3° C.) six weeks, given high-fat diet with 2% cholesterol but no choline. Note extensive deposition of fat in muscle fibres. (Oil red O. $\times 400$.)

FIG. 9.—Myocardium from same rat as Fig. 8 stained haematoxylin and eosin. (a) Note loss of striations and swelling of muscle fibres; occasional pyknotic nucleus. (b) More advanced stage, showing hyalinization and necrosis of muscle. ($\times 400$.)