THE MECHANISM OF TOXIC HEPATIC NECROSIS

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THE effect of many noxious influences on the liver is necrosis of the parenchymal cells, but the site of this necrosis varies. Most common around the central vein, it can also occur in the midzone or around the portal tract. The different lesions can be reproduced in animals by special diets or toxic chemicals and these experimental lesions have been widely studied in the hope of determining the mechanism of liver necrosis.

One theory, recently supported by Christie and Judah (1954) and Cameron (1954), attributes the effect of hepato-toxic agents such as carbon tetrachloride (CCl_4) to direct interference with cellular metabolism. Their predilection for certain areas of the lobule could be explained by metabolic differences between the cells of those parts (Mann, 1932 ; Deane, 1944). Another theory, advanced mainly by Himsworth (1947), attributes centrilobular necrosis to ischaemia of the cells around the central vein. Thus, CCl_4 necrosis is centrilobular because the sinusoids are narrowed by swelling of the parenchymal cells which reduces the blood supply to the central cells, whereas after allyl formate these cells are preserved because the sinusoids are dilated and the compound exerts a direct toxic effect on the periportal cells. This view, accepted by Williams (1948), Baxter (1948), Drill (1952) and Elias (1955) to explain toxic centrilobular necrosis, was extended by Himsworth (1947) and Glynn, Himsworth and Lindan (1948) to explain the massive hepatic necrosis and diffuse fibrosis produced by abnormal diets in rats. Glynn (1951), however, admits uncertainty about the mechanism of the initial swelling of the cells after CCl_4 which leads to the circulatory obstruction.

Very different methods have been used in attempts to substantiate these theories. Those seeking a direct effect of toxic chemicals on cellular metabolism have relied on tissue slice techniques or, more recently, on the study of particulate cell fractions. On the whole the chemical findings have been poorly correlated with the histological appearances and there are numerous discrepancies between the reported results. For instance, the O_2 consumption by liver slices from CCl_4-poisoned rats has been described as reduced by Christie and Judah (1954) and Niwa (1954), but as increased by Ennor (1942), Hove and Hardin (1951), Dianzani (1953) and Patwardhan, Ramalingaswarmi, Sriramachari and Patwardhan (1953). Recently a search has been made for specific lesions in the mitochondria. Morphological changes after CCl_4 and phosphorus have been shown by electron microscopy (Dianzani and Bahr, 1954). Dianzani (1954) considers the abnormal mitochondria to behave as if " uncoupling" had occurred. According to Christie and Judah (1954) CCl_4 produces a physical change in the mitochondria destroying their ability to retain necessary co-factors with consequent failure of

energy production. A similar view has recently been expressed by Dianzani (1955). The earliest histological sign of liver damage is in the mitochondria (Cameron and Karunaratne, 1936 ; Cameron, Milton and Allen, 1943), but it is difficult to decide whether this precedes or follows a vascular change. In either case it is surprising to find an increase in the adenosine triphosphate content of the damaged liver reported by Ennor and Stocken (1948) and confirmed by Rowles (1952).

Vascular changes in the liver during the production of necrosis have been studied in three ways—the distribution of India ink in the liver after injecting it into the spleen, the radiographic observation of the passage through liver of radio-opaque material injected into the portal vein, and direct observation of the trans-illuminated vessels of the liver.

Using the first method, Glynn and Himsworth (1948) claimed that swelling of the parenchymal cells restricted the intralobular circulation as early as 4 hr. after the subcutaneous injection of CCl_4 and that the effect persisted for many hours. Similar results were obtained by Andrews and Maegraith (1948) and by Baxter (1948) in pyridine necrosis although Stowell and Lee (1950) could not detect any difference in the size of the sinusoids in fixed material from CCl_4-poisoned mice. Glynn *et al.* (1948) also reported sinusoidal contraction in diet-induced fatty infiltration of the liver but this could not be confirmed by Fite (1954).

Daniel, Prichard and Reynell (1952*a*), who obtained variable results by the India ink technique, used the radiographic method and could not detect any alteration in the vascular pattern of the liver or rate of portal blood flow in either acute CCl_4 poisoning or fatty infiltration due to a high fat diet.

Loeffler and Nordmann (1925) transilluminated the liver edge in rats and mice exposed to chloroform. They observed constriction of the sinusoids lasting several hours. Wakim and Mann (1942) also found intra-hepatic vasoconstriction by this method, but only so long as CCl_4 was being inhaled. After exposure the normal appearance rapidly returned with later (20–24 hr.) obliteration of the sinusoids as necrosis became obvious. Seneviratne (1949) found only dilatation of the sinusoids during the first 8 hr. after the subcutaneous injection of CCl_4 followed by their compression and ultimate obliteration in the necrotic areas at 24 hr.

These three methods may be criticised for requiring anaesthesia and laparotomy which interfere with the temperature, and blood flow through the liver (Birnie and Grayson, 1952). Furthermore, in the transillumination method observation is confined to the edge of the liver, the behaviour of which may differ from that of the deeper parts (Daniel and Prichard, 1951 ; Elias, 1955).

The development of internal calorimetry by Grayson (1952) for the simultaneous determination of liver temperature and blood flow in the conscious animal suggested a fresh approach to this problem. It was hoped that by using this method to study the hepatic necrosis produced by a variety of toxic agents, information would be obtained which would help in deciding whether vascular or metabolic factors are more important in its production.

METHODS

Male rats (345 $\pm$ 45 g. body wt.) of the Porton strain fed on M.R.C. diet 41 (Bruce and Parkes, 1949) were used.

Centrilobular necrosis was produced in three ways. Dimethylnitrosamine (DMN), from the sample used by Barnes and Magee (1954), was injected subcutaneously as a 5 per cent (w/v) solution in 0·9 per cent (w/v) NaCl. CCl_4 (Analar) was either injected intra-

muscularly undiluted (Cameron and Karunaratne, 1936) or given by mouth as a 20 per cent (v/v) solution in arachis oil. Na fluoroacetate (Hicks, 1950) was injected intramuscularly as a 0·2 per cent (w/v) solution in 0·9 per cent NaCl.

Beryllium sulphate was injected into a tail vein as a 0·8 per cent (w/v) aqueous solution of $BeSO_4 . 4H_2O$; it produces necrosis which is initially midzonal (Scott, 1948; Aldridge, Barnes and Denz, 1949).

Periportal necrosis was produced by injecting redistilled allyl formate subcutaneously as a freshly prepared 1·5 per cent (v/v) solution in 0·1 M phosphate buffer (pH 7·4).

Recrystallised 3 : 5 dinitro-*o*-cresol (DNOC) (B.D.H.) was also used as a 1·0 per cent (w/v) solution in 0·1 M Na_2CO_3 injected intramuscularly. Ethyl ether (Analar) was injected intramuscularly. All these liquids except the last and allyl formate were warmed to 37° before injection.

The methods of internal calorimetry have been fully described by Grayson and his colleagues (Birnie and Grayson, 1952; Grayson, 1952; Grayson and Haigh, 1953; Grayson and Johnson, 1953; Ginsburg and Grayson, 1954; Johnson, 1954) and were used here with minor modifications (Stoner, 1954). Each recorder was used once and the instrument factor (F) determined in each case (Haigh, 1954). The mean value of F for 153 recorders was 118·2 ± 12·7 (S.D.). Results are expressed as the conductivity increment (δk) due to the flow of blood in the tissue around the recorder. This figure is the difference between the thermal conductivity of the living and dead tissue, measured as the square of the current, in amperes, required to raise the temperature of the heater filament 1° above that of the surrounding liver, divided by F for the recorder. A linear relationship between δk and liver blood flow over the physiological range has been established (Grayson, 1952; Grayson and Haigh, 1954; Grayson and Ginsburg, quoted by Johnson, 1954) even after the recorders have been embedded in the liver for some days (Grayson, 1954). Essentially the method measures the blood flow through a collar of tissue about 2 mm. radius and 2–3 mm. length around the recorder. There may be a considerable error in the "measurement of small percentage changes in flow at low initial blood flow levels" (Johnson, 1954). This observation arose from experiments on severe hypotension after elimination of the portal blood supply to the liver, but with normal initial blood flow levels it is unlikely that a major change in blood flow will be overlooked (Grayson, 1954). Provided that two other requirements are fulfilled the method should be adequate for the present purpose.

The tissue surrounding the recorder must be homogeneous (Grayson, 1952). This has been emphasised by the experience of Linzell (1953). Normal liver meets this requirement and the lesions produced in the present experiments are considered sufficiently diffuse for the method to be valid. Calder (1942) has shown the uniform way in which $CHCl_3$ injures the liver and this has been confirmed for CCl_4 and the other toxic agents in the lobe of the liver used in these experiments.

The successful use of the internal calorimetry method presupposes that the basal thermal conductivity, *i.e.*, of the "dead" liver, does not alter during the experiment (Grayson, 1952). The thermal conductivity values obtained at the end of the experiments show that this assumption is justified (Table I).

TABLE I.—*Thermal Conductivity* (k) *of Rat Liver after Death.*

Treatment.	Number of rats.	$k \times 10^4$ mean ± S.D.
Controls	28	11·8±1·23
Dimethylnitrosamine	24	11·9±0·93
Carbon tetrachloride	13	11·5±1·20
" " + arachis oil	13	11·7±0·96
Arachis oil	12	12·3±1·36
Sodium fluoroacetate	10	10·9±1·01
Beryllium sulphate	10	11·8±1·17
Allyl formate	16	11·6±1·14
Ethyl ether	9	11·2±0·90

The recorders were inserted into the right lobe of the liver (*pars centralis* of Gershbein and Elias, 1954) at laparotomy under ether anaesthesia on the day before the experiment began. After the operation 100 mg. oxytetracycline hydrochloride ("Terramycin—oral

drops "—Pfizer. Oral LD_{50} 720 mg. per 100 g. body wt. (P'an, Reilly, Halley, Richard, Pekich and Pollats, 1950)) were given by stomach tube and this standard dose was repeated each day after the last observation had been made. This prevented peritoneal infection during the period (< 5 days) of the experiment. The dose was based on the experience of Cheng (1954) in this laboratory. It could probably be reduced for the present purpose, but no ill effects were observed. Large doses of oxytetracycline cause liver damage (Lepper *et al.*, 1951), but no evidence of this was found in the controls given this dose. The rôle of infection in liver necrosis is debatable (György, 1951, 1952, 1954; Luckey, Reyniers, György and Forbes, 1954; Reynell, 1954; Eger, 1955), but the presence of an antibiotic in the present experiments did not appear to alter the pathological changes. Lindenbaum, White and Schubert (1954) have shown that only intravenous oxytetracycline chelates effectively with Be. The oral dose used here had no effect on the intravenous toxicity of $BeSO_4$.

The rats were placed in small wire cages (1·5 cm. mesh) while the observations were made. This took 5–10 min. and the animals usually remained quiet during this period. These cages did not restrict respiration and most of the rats could turn round in them. Hypothermia due to restriction and emotion (Ware, Hill and Schultz, 1947; Bartlett, Bohr, Helmendach, Foster and Miller, 1954) was thus avoided.

Each value of δk shown in the text is the mean of 2–4 readings. The liver temperature was measured to the nearest 0·1°.

At the end of the experiment the rat was killed with coal gas if it had survived the action of the toxic agent. After a short time for cooling, the thermal conductivity of the dead liver was measured. The position of the recorder in the liver was then examined and rats in which it was too near the surface or too close to major vessels were excluded. The I^2/θ test (Grayson and Johnson, 1953) was not used routinely, but a sufficient number of tests were made, both before and after death, to show that when the recorder was correctly placed the linear relationship between the square of the current (I) applied and the rise (θ) in temperature produced was unaffected by the poisons used. The recorder was finally removed and the liver around it fixed in Helly's fluid for histological examination.

Where possible results are expressed as the mean $\pm$ S.D. and a statistical comparison of the means made by Student's "t" test as modified by Fisher (1934) for small samples. The illustrations show the results in individual rats chosen because they exhibit most of the features being described.

RESULTS

Preliminary Control Observations

Since the observations usually extended over some days while the hepatic lesion was forming, some preliminary work was necessary to determine the range of the day-to-day variation in the liver temperature and blood flow. Some observations of this type were made by Birnie and Grayson (1952) and Grayson and Johnson (1953) but more detailed information was required for the present work.

Temperature

The mean liver temperature in 176 rats was 38·9° $\pm$ 0·5, in good agreement with the value given by Birnie and Grayson (1952). The mean liver temperature remained very constant over a number of days (Table II) but the temperature was consistently higher in the morning than in the afternoon. The average fall during the day, *i.e.*, between 9 a.m. and 6 p.m., was 0·7° $\pm$ 0·3 in 31 rats observed over a total of 76 days. As this appeared to be part of a diurnal cycle 12 rats were observed at intervals throughout a 24-hr. period. The liver temperature varied in a cyclical fashion (Fig. 1) the sequence of events being as follows.

From 9 a.m. the liver temperature fell steadily during the day while the rat was asleep. Towards evening, when the rat woke up, the temperature rose and reached its first maximum at about 10 p.m. The temperature then fell slightly

TABLE II.—*Mean Liver Temperature and Blood Flow* (δk) *in Rats observed between 9 a.m. and 6 p.m. on Succeeding Days.*

		Day of experiment.			
		1.	2.	3.	4.
I. Fed rats	Liver temp. °C.	38·5±0·3 (23)	38·6±0·4 (23)	38·6±0·3 (17)	38·4±0·3 (9)
	$\delta k \times 10^4$*	10·3±3·3 (17)	10·3±3·1 (17)	—	—
		9·5±2·7 (11)	9·5±2·2 (11)	8·8±2·6 (11)	—
		8·2±1·6 (6)	8·1±2·2 (6)	8·0±2·5 (6)	7·7±2·6 (6)
II. Rats fasted after day 1	Liver temp. °C.	38·6±0·3 (7)	38·4±0·4 (7)	38·5±0·4 (6)	—
	$\delta k \times 10^4$	8·6±2·2 (5)	8·5±1·3 (5)	7·4±1·6 (4)	—

* The values of δk in each line refer to the values obtained in the same group of rats. Number of rats shown in parentheses.

and later rose to a second maximum at 4–5 a.m. in all but 2 of the 12 rats. The temperature fall during the night was never as great as during the day so that the mean liver temperature between 8 p.m. and 5 a.m., 39·0° ± 0·26, was significantly ($P < 0{\cdot}01$) higher than the mean day (9 a.m.–6 p.m.) temperature, 38.6° ± 0·27. The first nocturnal peak, 39·3° ± 0·34, was usually higher than the second, 39·0° ± 0·34, but the difference was not significant in this small series ($P < 0{\cdot}1$; $> 0{\cdot}05$).

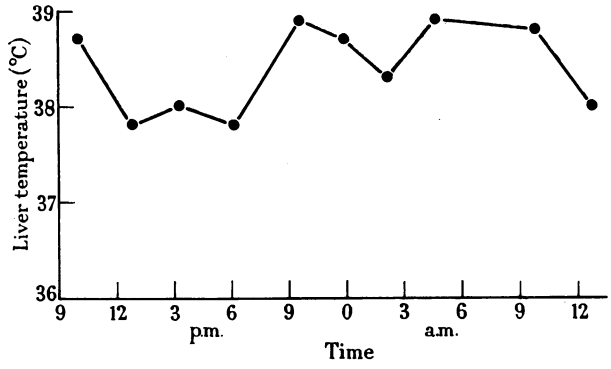


FIG. 1.—The cyclical variation in the liver temperature of a normal rat during a 24-hr. period.

These changes in liver temperature can be related to the diurnal rhythm of the liver constituents and blood sugar in the rat (Higgins, Berkson and Flock, 1932, 1933 ; Deuel, Butts, Hallman, Murray and Blunden, 1938 ; Pitts, 1942). The blood sugar level is highest at midnight, when it is about 10 per cent above the noon level. Similarly the liver glycogen level in the male is highest about

4 a.m. and lowest at about 4 p.m. These changes occur in rats feeding naturally and it can be shown that they are related to the feeding cycle rather than to the activity cycle. The variations in liver temperature, corresponding to changes in its metabolic activity, can also be most readily related to the two periods of food ingestion during the night.

Although these diurnal changes are determined by food intake they are not immediately abolished by fasting. The blood sugar cycle persists during fasts up to 48 hr. (Pitts, 1942). Similarly, the variations in liver temperature were not obliterated by fasts of this duration. This is important, since the food intake of the poisoned rats was reduced. In 7 rats food was removed after the first day's observations and the effect of a 48-hr. fast with free access to water is shown in Table II. The mean liver temperature fell slightly and the temperature fall between 9 a.m. and 6 p.m. was reduced from $0{\cdot}7° \pm 0{\cdot}24$ to $0{\cdot}5° \pm 0{\cdot}21$ but these differences were not statistically significant.

Blood flow

The mean liver blood flow ($\delta k \times 10^4$) from 145 observations on normal rats was $10{\cdot}1 \pm 3{\cdot}4$, again in good agreement with the resting value found for this species by Grayson and his colleagues. As pointed out by Birnie and Grayson (1952) and Grayson and Johnson (1953), the liver blood flow determined by this method shows little day-to-day variation (Table II) and remains very constant in an individual animal (Fig. 2). Very occasionally, after the recorder had been

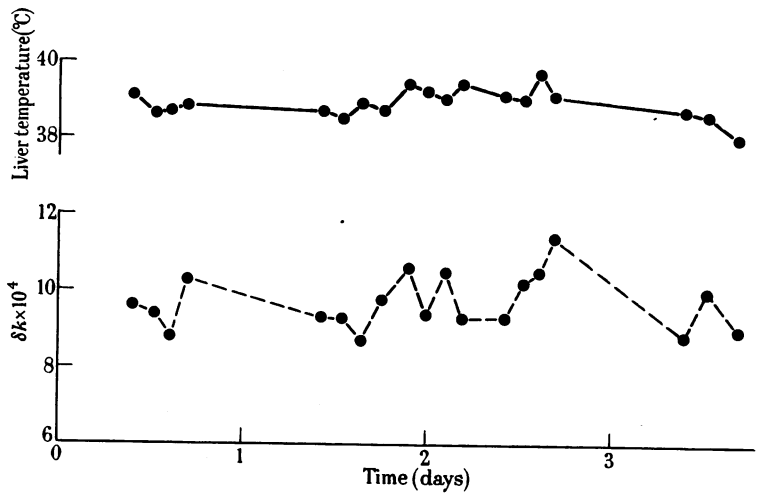


FIG. 2.—The liver temperature and blood flow (δk) in a normal rat over a period of four days.

in place for several days, the value of δk fell to a low level presumably because of fibrosis around the recorder. Temperature and blood flow in the liver are not necessarily correlated (Stoner, 1954) and there was no obvious diurnal variation in the blood flow. In the 12 rats where observations were made at intervals over 24 hr. the mean level of $\delta k \times 10^4$ between 9 a.m. and 6 p.m. was $9{\cdot}3 \pm 3{\cdot}3$ and

between 8 p.m. and 5 a.m. $10{\cdot}1 \pm 4{\cdot}1$, the difference not being statistically significant. The constancy of the liver blood flow during the day was also seen when the initial value of δk in the morning was compared with the mean value obtained during the rest of the day and with the maximum value recorded during that period (Table III). Fasting somewhat reduced the mean blood flow (Table II), but the difference was not statistically significant.

Histology

The appearance of the liver surrounding the wires 2 and 5 days after their insertion is shown in Fig. 3 and 4. There was very little acute inflammatory reaction to the presence of the wires in the liver, but after 3 days fibroblasts could usually be seen proliferating around them. The intensity of the fibroblast reaction varied considerably, but after 5 days the average width of this zone was about 0·3 mm. The parenchymal liver cells around the track appeared normal at all stages.

Metabolic inhibitors

To test this method of assessing the metabolic activity of the liver by determining its temperature the responses to Na fluoroacetate and DNOC were observed. The former should reduce the liver temperature by blocking the

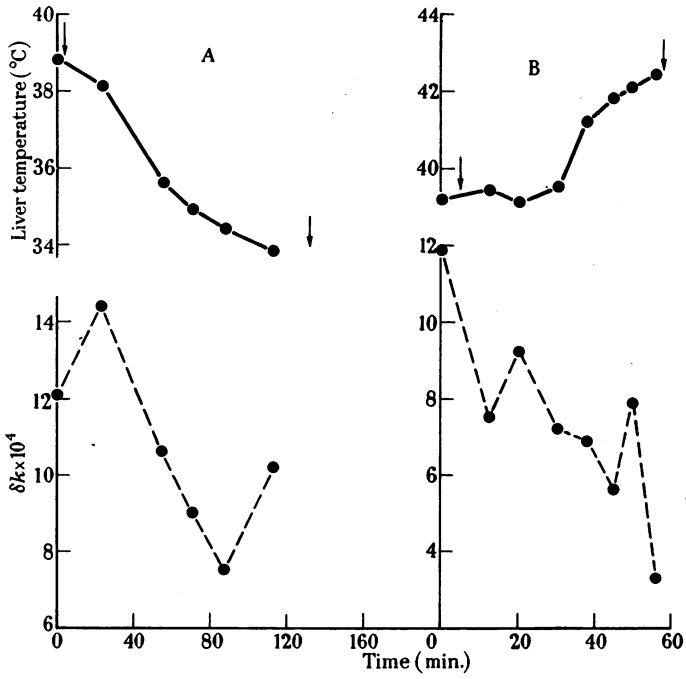


FIG. 5.—The effect of metabolic inhibitors on liver temperature and blood flow (δk) in the rat.
A. 0·5 mg. Na fluoroacetate per 100 g. body wt. injected intramuscularly at the first arrow.
B. 2·3 mg. dinitro-o-cresol per 100 g. body wt. injected intramuscularly at the first arrow.
Time of death indicated by the second arrow.

tricarboxylic acid cycle, whereas the latter should raise the temperature by stimulating oxidation without increasing the store of energy-rich phosphates. Fatal doses of these compounds produced these effects (Fig. 5).

" Toxic infarction "

To test the validity of the blood flow measurements under pathological conditions the phenomenon of " toxic infarction " was studied. This is a condition described by Cameron, Karunaratne and Thomas (1937) following the intraportal injection of CCl_4. A sufficiently uniform " infarction " of the liver was produced by injecting 0·1 ml. CCl_4 into as small a tributary of the portal vein as possible (Fig. 6.). The acute effect of this on the hepatic circulation of the anaesthetised rat after laparotomy has been studied by Seneviratne (1949) with the quartz rod technique. He reported a transient fall in liver blood flow with " extreme contraction of the sinusoids, small portal radicles and their terminal branches " lasting for a few minutes. The exactly similar effect on the conductivity increment

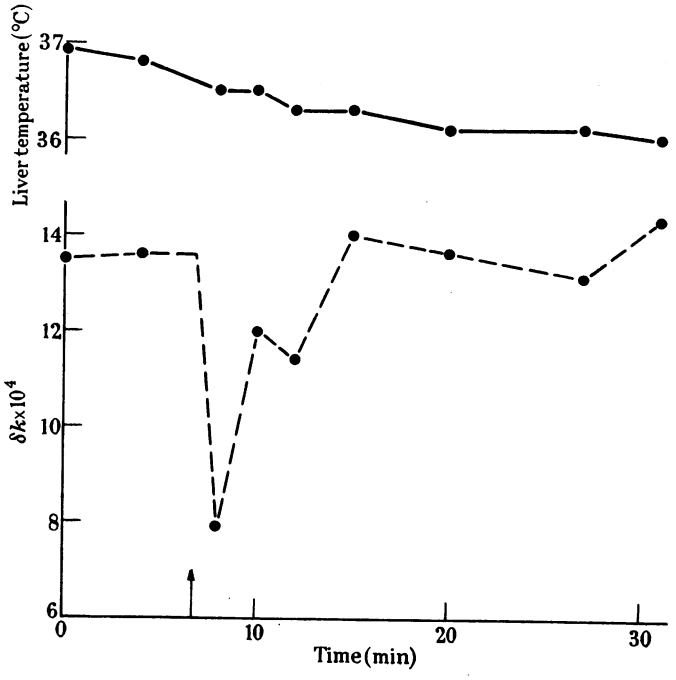


FIG. 7.—The effect of "toxic infarction" on the liver temperature and blood flow (δk) in the anaesthetised (ether) rat, 0·1 ml. CCl_4 injected into the portal vein at the arrow.

(δk) is shown in Fig. 7. The liver temperature underwent no dramatic change after the injection of CCl_4 but fell gradually until the rat was killed, in this case after 45 min. The low initial temperature and its subsequent fall in these experiments can, most probably, be attributed to the anaesthesia (ether), exposure of the liver and gradual deterioration of the preparation. In this instance the results of transillumination and internal calorimetry were in good agreement.

Centrilobular Necrosis

Dimethylnitrosamine $(CH_3)_2N.NO$

This compound was given subcutaneously in doses of 5·0 mg. per 100 g. body wt. This was a certainly fatal dose, the average survival time in those allowed to die being 56 hr.

The microscopical changes in the liver have been described by Barnes and Magee (1954). The first changes are seen after about 6 hr., when there is a decrease in the basophilia of the cells in the centre of the lobule and dilatation of the sinusoids. By 24 hr. there is well marked centrilobular necrosis and, at death, all cellular outline has been lost in the necrotic areas, which are well demarcated from the surviving cells (Fig. 8) and are frequently haemorrhagic.

This dose of DMN produced a very constant change in the liver temperature and blood flow (Fig. 9). During the first 8 hr. after the injection of DMN at about

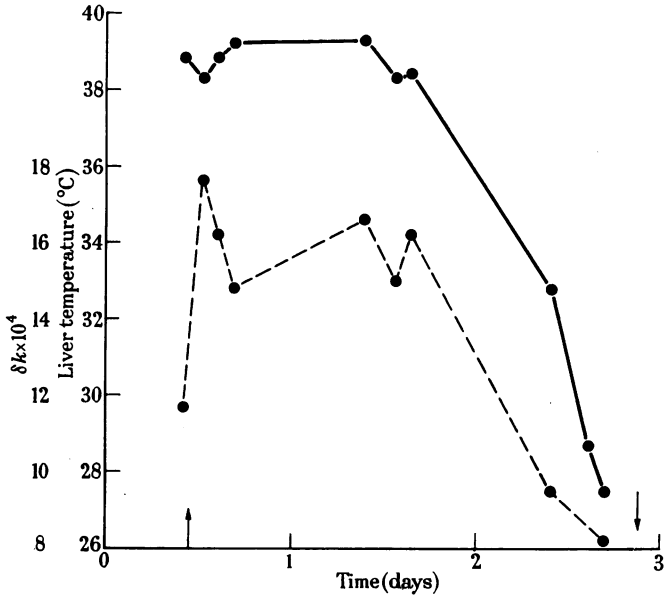


FIG. 9.—The effect of centrilobular necrosis on the liver temperature and blood flow (δk) in the rat. 5·0 mg. dimethylnitrosamine per 100 g. body wt. was injected subcutaneously at the first arrow. Time of death indicated by the second arrow.

10 a.m. on the first day of the experiment the liver temperature fell 0·8° ± 0·29. This was just significantly greater than in the controls. The hepatic blood flow, however, rose. Both the mean blood flow level during the day and the maximum blood flow recorded during that period were significantly higher than the pre-injection level (Table III). The first observations of liver blood flow following the injection of DMN were usually made after about 2 hr. when the rise in δk had usually begun. Some earlier observations were made, but there was no evidence that the rise in blood flow was preceded by a fall. After DMN, δk was

increased in all the rats, but in 2 out of 21 the rise was delayed until 24 hr. after the injection, the blood flow having remained within normal limits during the first 8 hr.

TABLE III.—*Effect of Certain Hepatotoxic Agents on the Liver Temperature and Blood Flow* (δk) *during the First 24 hr. after their Administration.*

Compound injected.	Fall in liver temp. between 0 and 8 hr. after injection °C.	Liver blood flow ($\delta k \times 10^4$). Before injection.	0–8 hr. after injection. Max.	0–8 hr. after injection. Mean.	24 hr. after injection.
DMN (5·0 mg./100 g. body wt. subcutaneously)	0·8 ±0·29† (20)	9·6±3·3 (19)	13·1±3·9‡ (19)	12·2±3·8† (19)	12·1±4·3* (14)
CCl_4 (0·1–0·8 ml./100 g. body wt. intramuscularly)	0·9 ±0·5† (14)	12·2±2·9 (12)	14·1±3·2 (12)	13·1±3·0 (12)	12·6±2·6 (12)
$CH_2F.COONa$ (0·2 mg./100 g. body wt. intramuscularly)	5·4 ±1·96§ (10)	10·9±4·6 (10)	11·3±3·9 (10)	10·2±3·6 (10)	10·3±4·3 (10)
$(C_2H_5)_2O$ (0·4 ml./100 g. body wt. intramuscularly)	5·0 ±2·3§ (9)	9·3±3·2 (8)	12·7±7·3 (8)	10·0±3·5 (8)	9·1±2·6 (7)
Controls	0·65±0·3 (76 observations in 31 rats)	9·9±3·3 (20)	10·6±3·5 (20)	9·9±3·3 (20)	9·6±3·0 (20)

Levels of *P* for the differences from controls (temp.) and from pre-injection level (δk).
* = < 0·10 > 0·05. † = < 0·05. ‡ = < 0·01. § = < 0·001.
Number of rats shown in parentheses.

Twenty-four hours after the dose of DMN the general condition of the rats was still fairly good and in 63 per cent the liver temperature was at its usual morning level but in the remainder it had begun to fall. This fall became general as the rats became ill and once this process had begun it continued until the animal died. Death usually occurred on the third day, when the liver temperature was about 25°.

In 78 per cent of the rats the liver blood flow was still increased 24 hr. after the injection of DMN and in half of these it was higher than on the previous day. In the others δk was about the same as it had been initially. This variability prevented the mean level at this time from showing a statistically significant difference from the mean initial level (Table III). Later, when the liver temperature fell, the blood flow through it decreased in a similar progressive fashion (Fig. 9).

Two additional rats were given 5·0 mg. DMN per 100 g. body wt. by stomach tube. The changes in liver temperature and blood flow were the same as when this dose was given subcutaneously.

Carbon tetrachloride

The histological changes in rat liver after a single dose of CCl_4 have been described by Cameron and Karunaratne (1936). For the first 2 hr. changes are confined to the mitochondria. After 5 hr. there is sinusoidal congestion, but the

parenchymal cells still appear normal. Centrilobular necrosis is present after 24 hr. The central cells show loss of structure, marked eosinophilia and nuclear changes, and these necrotic zones are bordered by the characteristic " balloon " cells produced by this agent (Fig. 10). The doses used here do not produce a fatal lesion ; the necrotic cells are removed and regeneration, well established after 3 days, is complete in about 14 days. The study of the changes during the development of necrosis has therefore been concentrated on the events of the first 24 hr.

In the first group of experiments undiluted CCl_4 was injected intramuscularly. To try to find conditions giving a typical response the dose was varied between 0·1 and 0·8 ml. per 100 g. body wt. in 10 rats feeding normally and in 5 fasted overnight. The outcome was uninfluenced by the nutritional state and there was no clear dose-response relationship within that range of intramuscular doses. Accordingly all the animals in which there was histological evidence for necrosis of half or more of the liver lobule (Fig. 10) have been grouped together (Table III).

The fall in liver temperature during the first 8 hr. after the administration of CCl_4 was significantly greater than the fall in the controls during that period. However, at 24 hr. the temperature was the same as it had been before the injection. The liver blood flow was not decreased during the production of the lesions, indeed the tendency was for it to increase although the differences between the means (Table III) were not statistically significant. Confirmation of this tendency was obtained by comparing the differences between the initial and maximum levels in the CCl_4-treated rats with the same differences in the controls. These differences were significantly ($P < 0{\cdot}02$) greater in the CCl_4-treated rats.

These results agree in part with those reported by Seneviratne (1949). He also found an initial increase in liver blood flow, but described a restriction of the circulation through the lobules when necrosis was established at 24 hr. This difference is of little importance from the point of view of the mechanism of the necrosis and the production of a more severe lesion in the presence of greater increase in liver blood flow will now be described.

CCl_4 is more effective when given by mouth (McCloskey and McGehee, 1950). In a second group of experiments CCl_4 was given by this route as a 20 per cent (v/v) solution in arachis oil, the dose being equivalent to 0·2 ml. CCl_4 per 100 g. body wt. Because Stohlman (1948) has shown that oral doses of corn and olive oils lower the rectal temperature the results were compared with those in controls given the same amount of arachis oil by mouth.

Oral doses of arachis oil affected both the temperature and blood flow of the liver (Table IV, Fig. 11). The liver temperature fell rapidly so that the reduction during the first 8 hr. was about twice that in untreated controls (Table III). At the same time δk was significantly increased. Twenty-four hours later normal values were obtained for the temperature and blood flow of the liver.

Addition of CCl_4 to the arachis oil greatly increased the immediate fall in liver temperature without altering the increased liver blood flow (Table IV). The temperature depression produced by 0·2 ml. CCl_4 per 100 g. body wt. given by mouth as a 20 per cent solution in arachis oil was greater than the sum of the separate effects of that amount of arachis oil by mouth and the same dose of CCl_4 intramuscularly. The fall in liver temperature was also more prolonged than after either substance alone (Fig. 11). Twenty-four hours after the administration of CCl_4 in arachis oil the liver temperature had regained its pre-injection level in only 2 of the 14 rats. By this time the δk level was the same as in the controls

TABLE IV.—*Effect of Carbon Tetrachloride in Arachis Oil and Arachis Oil alone on the Liver Temperature and Blood Flow* (δk) *during the First* 24 *hr. after Oral Administration.*

Compound injected.	Fall in liver temp. between 0 and 8 hr. after injection °C.	Liver blood flow ($\delta k \times 10^4$). Before injection.	0–8 hr. after injection. Max.	0–8 hr. after injection. Mean.	24 hr. after injection.
Arachis oil (1·0 ml./100 g. body wt. p.o.)	1·5±0·6 (12)	9·5±3·7 (12)	13·0±4·0† (12)	11·7±3·5 (12)	10·2±4·0 (12)
CCl_4 (0·2 ml./100 g. body wt. 20% (v/v) solution in arachis oil p.o.)	2·9±0·8* (14)	9·7±3·2 (13)	13·4±4·6† (13)	11·9±4·3 (13)	10·1±3·9 (13)

* = significantly different from " arachis oil " controls at $P < 0·001$.
† = significantly greater than the pre-injection level at $P < 0·05$.
Number of rats shown in parentheses.

(Table IV) and normal temperature levels were found after 48 hr. The further progress of these rats was not studied. Histologically, they showed much more severe hepatic necrosis than those given the same dose of CCl_4 intramuscularly despite the greatly increased hepatic blood flow during the development of the lesion.

Sodium fluoroacetate ($CH_2F.COONa$)

Although best known as an inhibitor of the tricarboxylic acid cycle (Peters, 1951), fluoroacetate in small doses which are not immediately fatal will produce

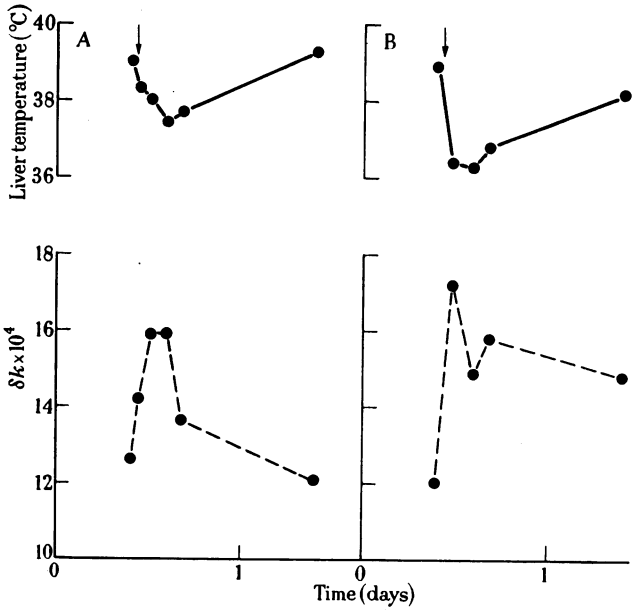


FIG. 11.—A comparison of the effects of oral doses of arachis oil with and without CCl_4 on the liver temperature and blood flow (δk) of the rat. A. 1·0 ml. arachis oil per 100 g. body wt. given by mouth at the arrow. B. 0·2 ml. CCl_4 per 100 g. body wt. given by mouth at the arrow as a 20 per cent (v/v) solution in arachis oil.

centrilobular hepatic necrosis (Hicks, 1950). The effect of rapidly lethal doses on liver temperature and blood flow has been described above. For the present purpose 0·2 mg. Na fluoroacetate per 100 g. body wt. was injected intramuscularly. This dose, close to the M.L.D., produced occasional convulsions, but all the 10 rats used survived more than 24 hr. after its injection. All showed centrilobular necrosis, but in comparison with the other agents used this was never severe (Fig. 12).

Liver temperature and blood flow were observed for the first 24 hr. after the injection of fluoroacetate (Table III). The liver temperature fell steeply so that after 3¼ hr. it was 4–5° below the pre-injection level. In 7 of the 10 rats it was still depressed after 24 hr. and in 4 of these the liver temperature was still falling. Despite this striking effect on liver temperature there was no definite alteration in liver blood flow (Table III) although in individual rats the fluctuations were often greater than in the controls.

"Midzonal" Necrosis

"Midzonal" necrosis of the liver was produced by the intravenous injection of 0·11–0·15 mg. Be^{2+} per 100 g. body wt. This dose, twice the LD_{50} (Aldridge *et al.*, 1949), was usually fatal, the survival time being about 58 hr. The histological changes in the liver were similar to those described by Scott (1948) and by Aldridge *et al.* (1949). With this dose there was definite zonal hepatic necrosis after 24 hr. From being initially a zonal lesion the necrosis spread so that at death practically all the liver cells were affected (Fig. 13).

Be^{2+} in a dose of 0·11–0·15 mg. per 100 g. body wt. produced very constant changes in the liver temperature and blood flow (Fig. 14). Almost immediately (10 min.) after the injection 80 per cent of the rats showed an increase in δk. This was not usually maintained and, as it was sometimes seen after the intravenous injection of equivalent volumes of 0·9 per cent NaCl, may well be non-specific.

EXPLANATION OF PLATES

All sections (5μ) stained with haematoxylin and eosin.

FIG. 3.—Section of the liver of a control rat cut parallel to the recorder wires after they had been in place for two days; shows the central track left by the wires and normal liver parenchyma on either side of it. (× 50.)

FIG. 4.—Section of the liver of a control rat cut at right angles to the recorder wires after they had been in place for five days. There is some fibroblast proliferation around the site of the wires with normal liver parenchyma beyond. The diameter of the black circle corresponds to 4 mm., being the diameter of the cylinder of tissue in which the blood flow is measured. (× 17.)

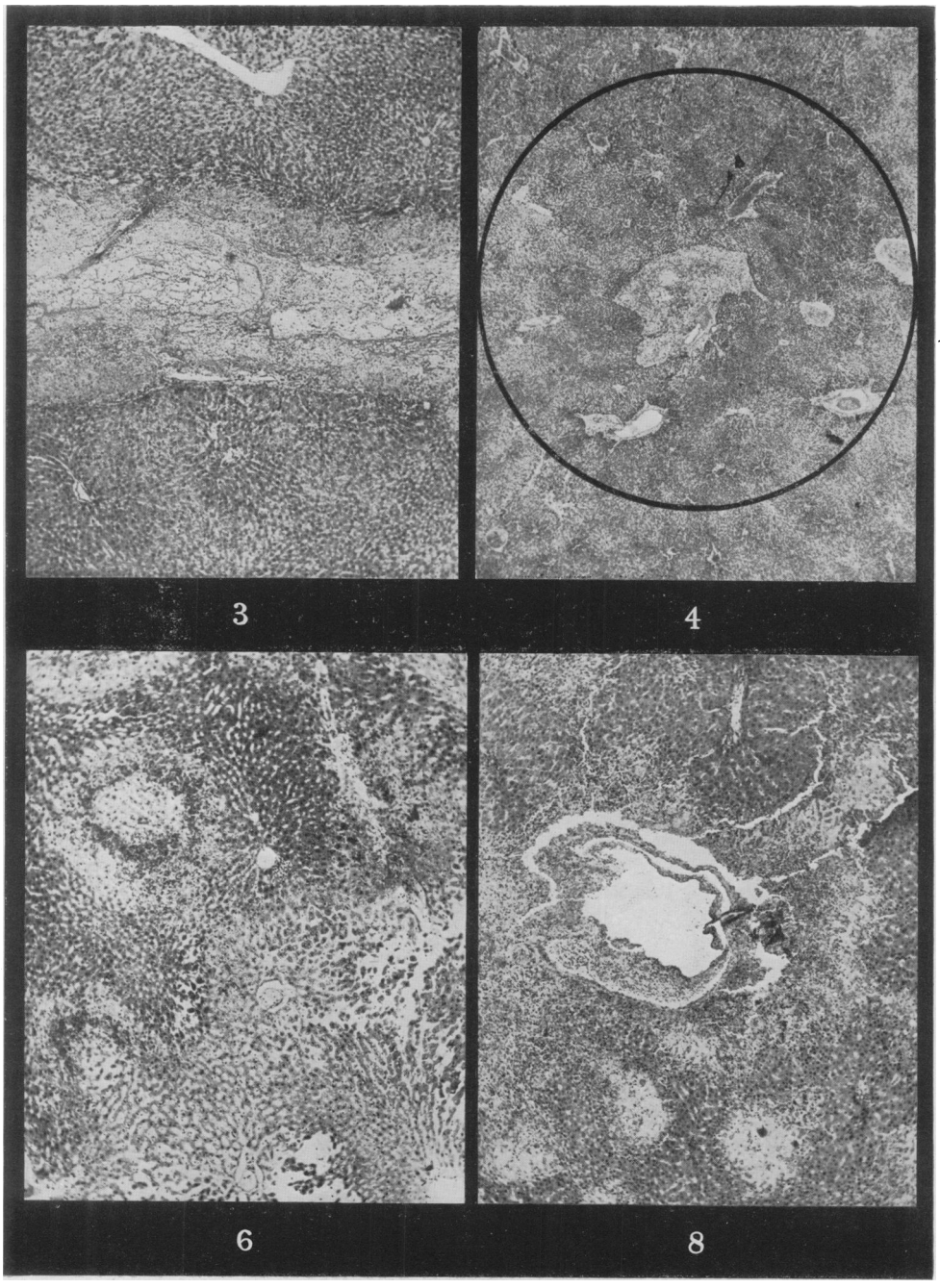
FIG. 6.—"Toxic infarction." Section of the liver of a rat killed 45 min. after the injection of 0·1 ml. CCl_4 into a small branch of the portal vein under ether anaesthesia. (× 50.)

FIG. 8.—Centrilobular necrosis. Section of the liver of a rat dying 54 hr. after the subcutaneous injection of 5·0 mg. dimethylnitrosamine per 100 g. body wt. The section has been cut at right angles to the recorder, the position of which is shown by the central space. (× 50.)

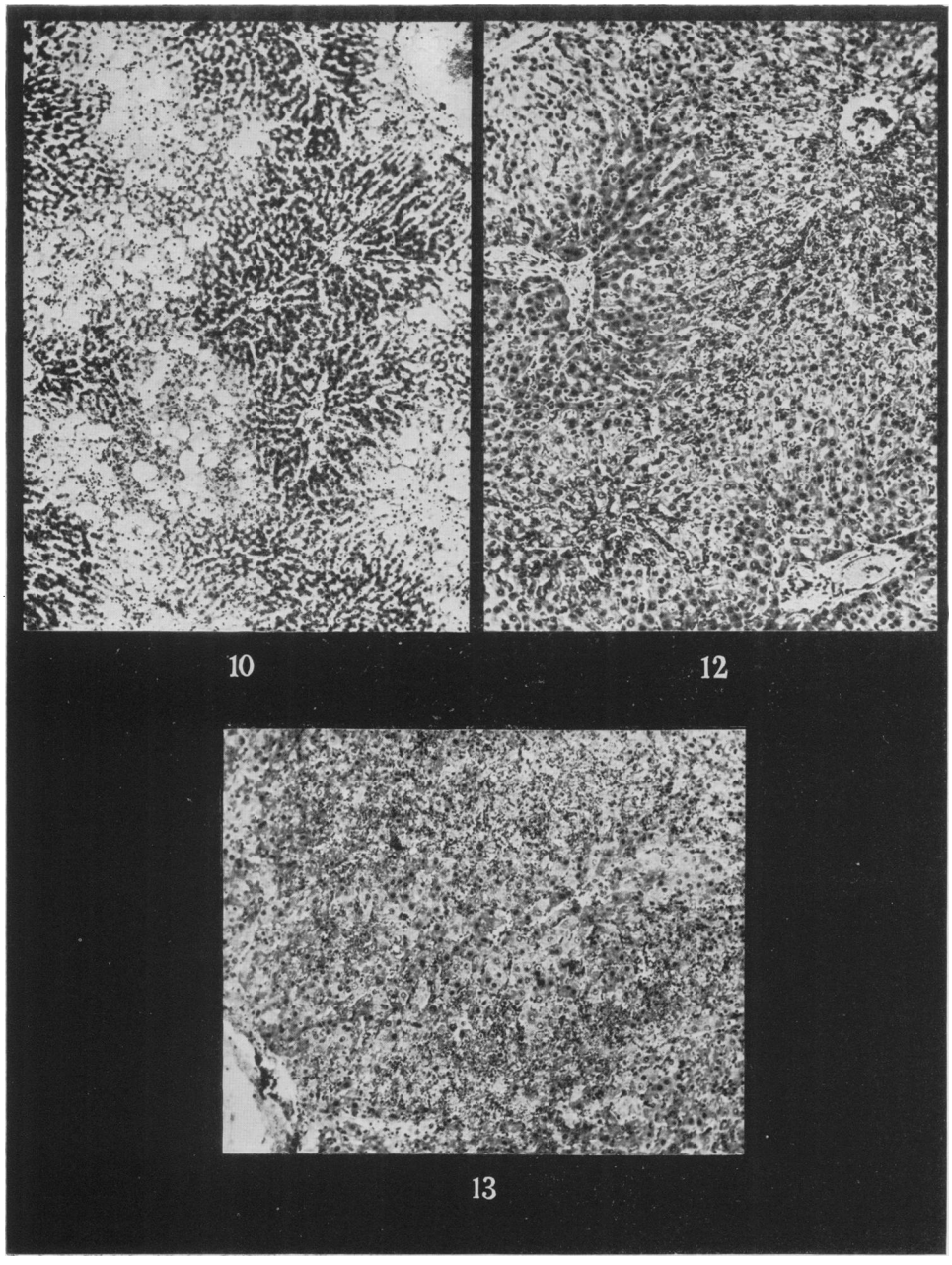
FIG. 10.—Centrilobular necrosis. Section of the liver of a rat killed 24 hr. after the intramuscular injection of 0·2 ml. carbon tetrachloride per 100 g. body wt. The section has been cut at right angles to the recorder, the track of which is seen in one corner. (× 50.)

FIG. 12.—Centrilobular necrosis. Section of the liver of a rat killed 24 hr. after the intramuscular injection of 0·2 mg. Na fluoroacetate per 100 g. body wt. This rat showed the most severe hepatic lesion produced by this agent. (× 100.)

FIG. 13.—"Midzonal" necrosis. Section of the liver of a rat dying 55 hr. after the intravenous injection of 0·15 mg. Be^{2+} per 100 g. body wt. The section has been cut at right angles to the recorder, the track of which is seen in one corner. (× 100.)



Stoner.



10

12

13

The blood flow changes in the first hour after Be have therefore been excluded from the statistical examination of the δk values (Table V), which shows that there was no significant change in δk during the first 32 hr. after the injection of Be.

TABLE V.—*Effect of* 0·11–0·15 *mg.* Be^{2+}/100 *g. body wt. intravenously on the Liver Temperature and Blood Flow* (δk) *in the Rat.*

Liver temperature °C.			Liver blood flow ($\delta k \times 10^4$).		
Mean fall 0–8 hr. after Be.	Mean temp. 0–8 hr. after Be	Mean temp. 24–32 hr. after Be.	Before Be.	Mean 1–8 hr. after Be.	Mean 24–32 hr. after Be.
0·7±0·35 (14)	38·9±0·29 (14)	37·9±0·62* (14)	9·5±2·7 (9)	10·7±2·1 (9)	9·4±3·2 (9)

* = Significantly different from the mean temp. 0–8 hr. after Be at $P < 0{\cdot}001$.
Number of animals shown in parentheses.

There was no immediate effect on liver temperature, its fall during the first 8 hr. (Table V) being the same as in the controls. The temperature was still well maintained on the day after the injection, but at a lower level than before. Comparison of the mean temperatures during these periods showed a significant difference and this drop in liver temperature after 24 hr., corresponding to the appearance of zonal necrosis, was the first sign of damage due to Be. Subsequently, with the increasing necrosis, the liver temperature fell progressively as the rat became moribund. This was accompanied by a reduced blood flow, but it was noticeable that there was no appreciable change in δk until there had been a marked fall in liver temperature (Fig. 14). In this the Be-treated rats differed from those given DMN for in the final stage of that illness liver temperature and blood flow fell together. The character of the change in hepatic blood flow was the same as

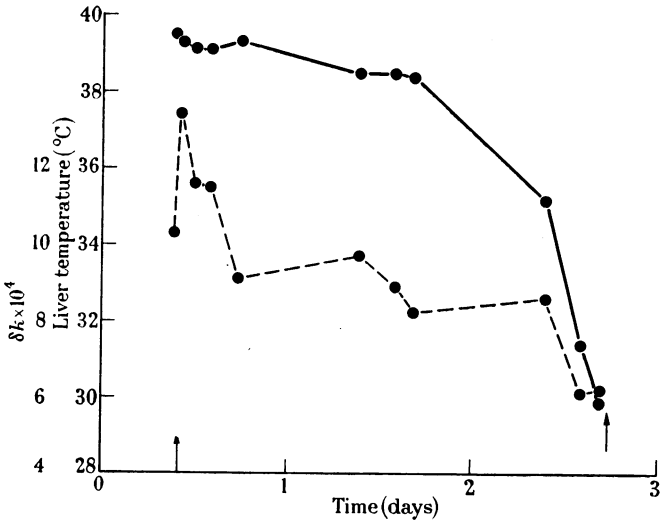


FIG. 14.—Effect of " midzonal " necrosis on the liver temperature and blood flow (δk) in the rat: 0·15 mg. Be^{2+} per 100 g. body wt. was injected intravenously at the first arrow as an aqueous solution of $BeSO_4$. Death occurred at the second arrow.

that described by Cheng (1956) using the quartz rod and flash photomicrographic techniques. He found a progressive contraction of the sinusoids beginning after 3 hr. with extensive sinusoidal obliteration after 16 hr.

Periportal Necrosis

Allyl formate (HCOO.CH$_2$CH : CH$_2$)

Allyl formate has been commonly used to produce periportal necrosis since its properties were first noted by Piazza (1914). Eppinger (1937) used it in the development of his concept of " serous inflammation ", for he considered that it was primarily a vascular poison and that the periportal lesion was secondary to this. Heinemann (1937) maintained that allyl formate did not produce hepatic necrosis in the rat, but it would seem from the work of Rosin and Doljanski (1946) and from the present experiments that her results were due to an unfortunate choice of doses.

TABLE VI.—*Average Survival Time and Histological Picture after Intramuscular Doses of Allyl Formate in the Rat.*

Dose. ml. per 100 g. body wt. intramuscularly.	Survival time (hr.). Mean ± S.D.	Histology.
0·003	Indefinitely	Congestion of the sinusoids with increased eosinophilia and some pyknosis of the nuclei in the parenchymal cells around the portal tracts. Signs of recovery after 48 hr.
0·006	21·3±12·8	Oedema of the portal tracts with an excess of inflammatory cells. Necrosis of the periportal cells seen as early as 8 hr. after the injection and in rats surviving more than 24 hr. formed a band about four cells wide. Beyond this zone there was an area of increased cytoplasmic eosinophilia and vacuolation.
0·008 0·009	5·6± 1·9 5·4± 1·5	More severe changes were seen at an earlier stage. There was marked eosinophilia, pyknosis and loss of nuclei with areas of coagulative necrosis occupying up to three quarters of the liver lobule.
0·015	1·5± 0·4	—

The dose used varied between 0·003 ml. and 0·015 ml. per 100 g. body wt., the M.L.D. for this strain of rat being about 0·005 ml. per 100 g. body wt. The average survival time and the histological changes, in haematoxylin and eosin preparations, following the different doses are given in Table VI. For any given dose the histological changes were more variable than after the other compounds used. The speed with which periportal necrosis developed after the larger doses was very remarkable and was accompanied by equally dramatic changes in both liver temperature and blood flow.

The most constant effect of the injection of allyl formate was a fall in liver temperature (Fig. 15), and after the larger doses this continued until the rat died. After 0·003 ml. allyl formate per 100 g. body wt. the fall continued for about 2 hr., after which it slowly recovered so that normal values were obtained on the following day. This effect was so constant that when the mean fall in liver temperature during the first 2 hr. was plotted against the logarithm of the dose a clear log. dose-response relationship was seen for the dose range (0·003–0·009 ml.

per 100 g. body wt.) in which observations were made (Fig. 16). With all the doses the fall in temperature was significantly greater ($P < 0{\cdot}001$) than in the controls.

The changes in liver blood flow were more complex. The percentage change in δk 2 hr. after the injection of allyl formate varied both in size and direction, and there was no dose-response relationship (Fig. 16). Results in individual rats showed that the predominant effect was a fall in δk. After fatal doses the hepatic blood flow was invariably decreased shortly before death, usually having fallen steeply, as in Fig. 15A, to very low levels. This was not their initial effect, however, for there was nearly always a period commencing about 30 min. after the injection

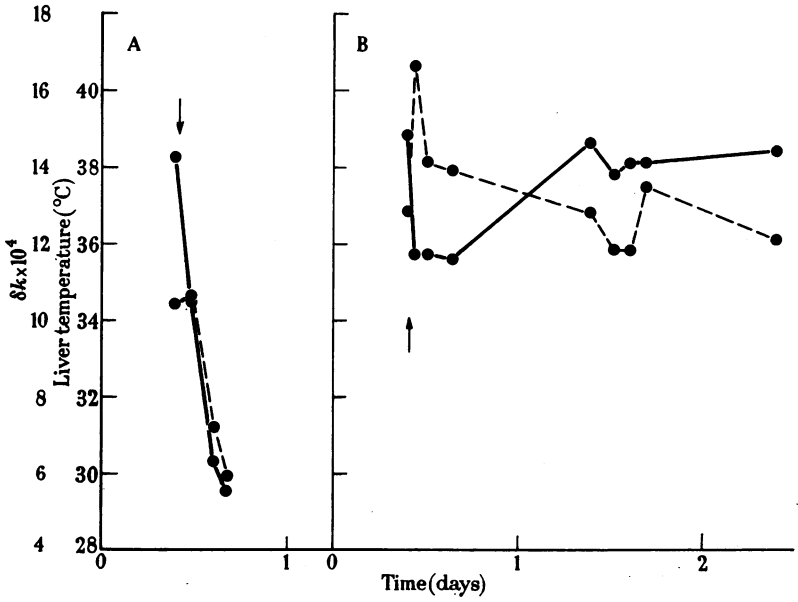


FIG. 15.—Effect of periportal necrosis on the liver temperature and blood flow (δk) in the rat. A. 0·006 ml. allyl formate per 100 g. body wt. injected intramuscularly at the arrow. The rat died during the night. B. 0·003 ml. allyl formate per 100 g. body wt. injected intramuscularly at the arrow.

and lasting 1–2 hr. when the blood flow was increased. This was a transitory effect, usually complete by 2 hr. after the injection, which appeared to correspond to a phase of generalised vasodilatation, for the ears showed a pink or purplish flush at that time. Since the liver temperature fell *ab initio* this means that the temperature had fallen several degrees (Fig. 15 and 16) before there was any significant fall in liver blood flow. This dissociation of the temperature and blood flow effects was best seen in rats given 0·003 ml. allyl formate per 100 g. body wt. These animals showed a proportionate fall in liver temperature (Fig. 16), but the change in δk was usually confined to the transitory rise, after which the blood flow remained within normal limits (Fig. 15). Very little necrosis, at the most a few scattered foci, was seen in rats given this dose. The characteristic necrotic lesion was only seen after the larger doses, which depressed both the temperature and the blood flow.

The Effect of Ethyl Ether

To obtain further control data the effect of ethyl ether was observed since it does not cause hepatic necrosis yet has pharmacological properties in common with $CHCl_3$ and CCl_4.

Rats were given 0·4 ml. ether per 100 g. body wt. intramuscularly. This dose did not produce complete anaesthesia, but made the animals stuporose for 1–2 hr. All except 1 of 15 rats recovered. Histological examination of the liver taken from rats killed 24 hr. after this dose of ether showed congestion of the sinusoids.

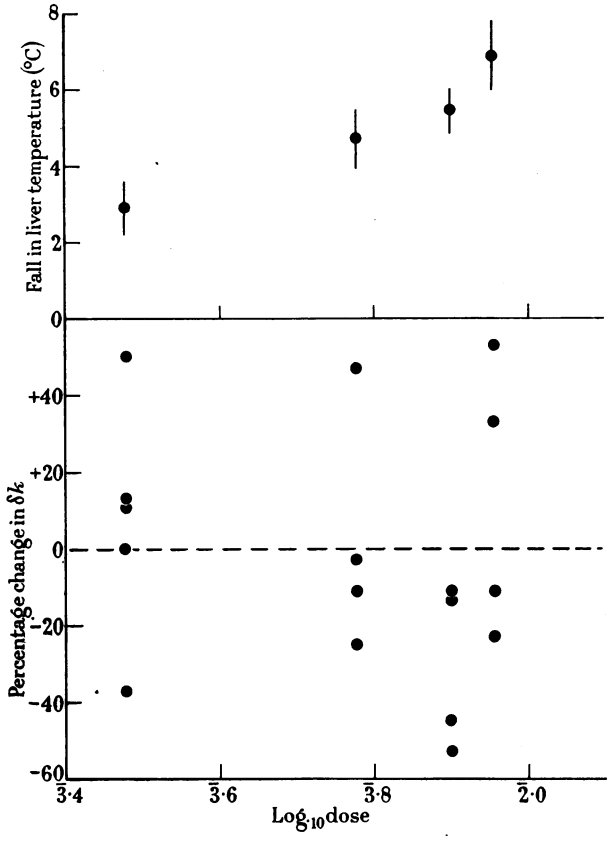


FIG. 16.—The upper part of the Figure shows the fall in liver temperature during the first 2 hr. after different doses of allyl formate in the rat. The points correspond to the mean values and the standard deviation is indicated by the vertical line. The lower part of the Figure shows the percentage change in δk for the liver 2 hr. after different doses of allyl formate. Each point corresponds to an individual rat.

There were numerous polymorphs in the sinusoids and blood vessels and in the track of the recorder. This was probably due to a general leucocytosis in response to the tissue damage at the injection site, for the leg was oedematous and the muscle showed a patchy necrosis. No definite hepatic necrosis was seen. Occasionally the cell and nuclear outlines were blurred in a zone about one cell thick around some of the centrilobular veins.

The changes in liver temperature and blood flow are summarised in Table III. A fall in liver temperature was constant, and except in the rat which died the minimum was reached after about 2 hr., a gradual rise to normal following up to 24 hr. The changes in δk were not so constant. In 4 rats it rose significantly, in 2 it fell significantly and remained unchanged in the remaining 2. These changes cancelled out in the averages (Table III) which showed no significant deviation. In the rat which died the liver temperature and δk fell progressively.

DISCUSSION

If these results can be accepted as indicating true changes in liver metabolism and blood flow it should be possible to draw some conclusions about the mechanisms involved in the production of the lesions studied.

Previous workers are agreed that the temperature of the liver is conditioned by its metabolism, and this view is strongly supported by its diurnal fluctuation, which can be easily related to biochemical events in the liver and by observing the predicted effects of DNOC and fluoroacetate.

Grayson and his colleagues have shown that an accurate picture of the changes in liver blood flow during physiological experiments can be obtained by internal calorimetry. It is unlikely that a major change in blood flow, such as might occur under pathological conditions, would be overlooked, particularly as the lesions did not alter the basal thermal conductivity of the liver or the I^2/θ relationship. The results of internal calorimetry cannot be directly correlated with those of dye injection or transillumination since each method measures a different aspect of blood flow. Many workers have found the simple dye injection method unreliable. In its most refined form as used by Daniel *et al.* (1952*a*) it gives information mainly about the structure of the blood vessels and the rate of flow in them, as for instance in these authors' study of the cirrhotic liver (1952*b*). The work most relevant to the problem of hepatic necrosis comes from the use of the transillumination technique and some attempt at correlation must be made. At first, when a direct comparison with transillumination was made under similar conditions of anaesthesia and laparotomy, *i.e.*, in toxic infarction, closely similar results were obtained. Later, discrepancies appeared which must be considered. Two features of internal calorimetry should be re-emphasised at this point. Firstly, it assesses the blood flow through a small collar of tissue around the recorder and the effects observed are due to changes in those cells. Secondly, it does not record the flow at any instant but the mean flow during the period required to take a reading, about 1 min.

The position of the recorder in the depths of the liver may explain why no evidence was found, either in the controls or in the rats given toxic agents, of the occasional restriction of the circulation through the superficial regions of the liver described by Daniel and Prichard (1951) using a radio-opaque dye and seen by most users of the transillumination technique. Other factors may be involved in this, for both methods differ from internal calorimetry in requiring anaethesia and laparotomy during the experiment. These factors may explain the other discrepancies. It would seem from the results of Seneviratne (1949) with CCl_4 and of Cheng (1956) with Be compounds that the transillumination method is more sensitive than internal calorimetry. However, it may be that the circulation in the damaged liver is more sensitive than in the normal liver to the depressant

effects of anaesthesia and laparotomy. This idea is supported by the protocols shown in Table VII. It is thought that by the elimination of these factors internal calorimetry may give the most faithful picture of the changes in liver blood flow.

TABLE VII.—*Effect of Nembutal and Laparotomy on the Liver Temperature and Blood Flow* (δk) *in a Rat given* CCl_4.

	Liver temp. °C.	Liver blood flow ($\delta k \times 10^4$).
I. CCl_4-treated rat :		
Before CCl_4	39·4	8·3
24 hr. after CCl_4	36·4	8·1
After operation	34·2	3·1
II. Control (no CCl_4) :		
Before operation	38·9	9·9
After operation	36·1	9·7

CCl_4 was given in a dose of 0·2 ml. per 100 g. body wt. by mouth as a 20 per cent solution in arachis oil 24 hr. before operation.

The immediate effect of all the compounds causing centrilobular necrosis was accentuation of the day-time fall in liver temperature, indicating depression of the liver metabolism. To this extent the results support the " metabolic " theory of the production of centrilobular necrosis. However, although with CCl_4 the greater the fall in temperature the more severe the necrosis, among the different compounds there was no correlation between the magnitude of this effect and the size or histological appearance of the lesion. Fluoroacetate with the greatest effect produced least necrosis. The marked effect of this compound on liver temperature was expected, since it gives rise to a potent inhibitor of cell oxidation. DMN, with little effect on the O_2 uptake of liver slices (Vandekar, personal communication), only slightly increased the fall in temperature yet produced the most severe lesion in the liver. The necrosis produced by DMN was fatal and towards the end both liver temperatures and blood flow fell dramatically. Necrosis preceded these terminal changes, which might reflect either the final failure of the liver or the moribund state of the rat. These alternatives could not be differentiated and both factors may be involved.

These experiments on centrilobular necrosis give no support to the "ischaemia" theory of its production. With all the agents studied the hepatic blood flow was either unchanged (fluoroacetate) or increased (DMN and CCl_4) during the production of the lesion. The increase was most marked after DMN and with the particular compounds used was positively correlated with the severity of the lesion. With CCl_4 the more severe lesion was produced in the face of the large increase in blood flow due to the arachis oil. There is no reason to suppose that these changes in δk have arisen other than by the action of these compounds on the liver tissue around the recorder.

The centrilobular necrosis produced in these experiments cannot be attributed to circulatory disturbances and is thought to be due to direct interference with the metabolism of the affected cells. It is not possible from these experiments to say much about the metabolic reactions concerned. It is clear that centrilobular necrosis was not due to simple interference with the oxidative mechanisms of the affected cells. This is shown by the lack of correlation between the fall in liver temperature produced by a compound and its necrotic action and also by the fact

that ether markedly lowers liver temperature without producing necrosis. The nature of the primary biochemical disturbance, which may be different in each case, and the possibility that the localisation of the lesion is due to biochemical differences between the central and peripheral cells await further study.

A possible explanation of the " midzonal " necrosis produced by Be compounds has recently been proposed by Cheng (1956) working in this laboratory. He considers that after injection Be circulates as a colloidal compound which is removed by the Kupffer cells. These undergo necrosis and this process spreads, with diffusion of the toxic agent, to the adjacent liver cells to give the histological appearance seen after about 24 hr. The circulatory disturbance in the liver is thought to be secondary to these changes. The results obtained with internal calorimetry fit Cheng's theory very well. The latent period before significant change in liver temperature agrees with the idea that alterations in the liver parenchyma only occur after destruction of the Kupffer cells, for these must contribute very little to heat production by the liver. The secondary nature of the decrease in liver blood flow is clearly shown by this method.

Neither of the two explanations (Eppinger, 1937 ; Himsworth, 1947) proposed for the periportal necrosis produced by allyl formate is in complete agreement with the results of internal calorimetry. Allyl formate is certainly more than a vascular poison. This is shown by the log. dose-response relationship for the depression of the liver temperature, and these results support the view of Fleckenstein (1944) and of Kessel, Körtge and Pezold (1954) that it is a general metabolic poison. Indeed, this would seem to be its main action, since the fall in liver temperature is independent of any changes in blood flow. These latter are complex, being the result of opposing forces. While it would be unwise to say that the changes in liver blood flow are entirely secondary to the alteration in its metabolism, the fall in blood flow probably follows the general metabolic depression. If the dose of allyl formate is sufficient this effect interferes with the primary rise in flow. This primary rise seems to be associated with a generalised vasodilator action of the compound. It is, however, doubtful if the dilatation of the sinusoids can be considered to protect the central cells for, after necrotising doses of allyl formate, the flow through them is very slow. On the other hand, does the fall in blood flow help to produce the periportal lesion, for definite necrosis was only seen when this occurred? These data do not provide a simple answer, but the metabolic effects of allyl formate seem to outweigh the vascular ones and it appears reasonable to attribute the periportal necrosis to them.

The general conclusions from this study of the effect of a range of hepatotoxic agents on the temperature and blood flow of the liver are that the lesions they produce are due to interference with the metabolism of the affected cells and that any changes in blood flow are either secondary to this or due to other causes.

SUMMARY

The changes in liver temperature and blood flow during the production of hepatic necrosis by dimethylnitrosamine, carbon tetrachloride, sodium fluoroacetate, beryllium sulphate and allyl formate have been followed in the rat by the method of internal calorimetry to determine whether this necrosis is due to an alteration in the blood supply to the liver cells or to direct interference with their metabolism.

The suitability of the method was tested by observing the effect of 3,5-dinitro-*o*-cresol and sodium fluoroacetate on liver temperature. This was increased by the first and reduced by the second compound in accordance with their known metabolic effects. The changes in liver blood flow in " toxic infarction " produced by the intraportal injection of carbon tetrachloride agreed with Seneviratne's results using the transillumination method.

In the normal rat the liver blood flow remained relatively constant but the temperature of the liver showed a diurnal cycle.

Substances causing centrilobular necrosis immediately increased the day-time fall in liver temperature. This effect was minimal with dimethylnitrosamine and maximal after fluoroacetate. The liver blood flow during the production of the necrosis was unchanged by fluoroacetate but increased with the other compounds. The necrosis produced by the doses used was in the order dimethylnitrosamine > carbon tetrachloride > sodium fluoroacetate.

Fatal doses of beryllium sulphate had no immediate effect on liver temperature or blood flow but after 24 hr., when necrosis was present, the temperature in the liver fell followed by a reduction in its blood flow.

The main effect of allyl formate was on the liver temperature, which fell in proportion to the log. dose over the dose range used. Small doses increased the blood flow, but with larger doses there was a secondary fall in liver blood flow which, after high doses, frequently replaced the early increase.

The intramuscular injection of ethyl ether caused a marked fall in liver temperature without producing necrosis.

These findings are considered to support the view that the lesions of toxic hepatic necrosis are not due to interference with the blood supply to the liver but result from a direct interference with the metabolism of the liver cells.

My thanks are due to Dr. J. M. Barnes for his interest in this work, to Mr. H. McKinnon for his care in making the recorders, and to Mr. R. F. Legg for taking the photomicrographs. The beryllium sulphate was kindly supplied by Murex Ltd., and the sodium fluoroacetate by Associated Fumigators Ltd.; and my thanks are also due to Pfizer Ltd., for supplying part of the terramycin.

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