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CONTROLLED INDUCTION OF CIRRHOSIS IN THE RAT

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Summary.—The production of experimental cirrhosis in the rat, most commonly by multiple doses of carbon tetrachloride (CCl_4), is a difficult process with a low yield of "cirrhosis" of widely varied histology. This is due to an unpredictable variation in the response of the rat liver to CCl_4, and the lack of a reliable method of monitoring the rapidly changing liver damage with each dose. A simple non-invasive method is described in which the daily body weight change of the rat in response to weekly intragastric doses of CCl_4 has been shown empirically to sufficiently reflect the state of the liver as to enable each dose of CCl_4 to be calibrated by the weight change of the previous dose. The death rate is markedly reduced and a critical level of liver damage can be maintained. This improved control over liver damage has made it possible to produce a high yield (72%) of a standardized decompensated micronodular cirrhosis with 8–10 doses of CCl_4. Under these weight-calibrated conditions this point is determined non-invasively by using a visual grading of a critical level of ascites estimated during light halothane/oxygen anaesthesia to relax the abdominal musculature.

ALTHOUGH ALCOHOL is the main cause of cirrhosis in the Western world (Lelbach, 1975), it is now generally accepted that alcohol alone is not an adequate enough hepatotoxin for the production of micronodular cirrhosis within the lifespan of the smaller laboratory animal (Rubin and Lieber, 1975; Patek, Bower and Sabesin, 1976). A great many agents have been used over the years as hepatotoxins in experimental animals, but for most practical purposes the commonest method of attempting to produce experimental cirrhosis today is to use multiple doses of carbon tetrachloride (CCl_4), given either s.c. or by inhalation (McLean, McLean and Sutton, 1969), in the rat. However, in spite of the fact that CCl_4 has been used for this purpose for over 60 years (Feissinger, Wolff and Blum, 1922), the production of a consistent yield of experimental cirrhosis, in particular of the decompensated micronodular form, has remained to this day an unpredictable and discouraging endeavour (Daniel, Prichard and Reynell, 1952; Hays and Okumura, 1967; McLean *et al.*, 1969; Wood *et al.*, 1979).

The problem stems from two sources; the *mechanism* of the production of cirrhosis by hepatotoxins, and the unpredictable *variation* of the hepatic response to CCl_4 in the rat, both between the rats in a group and within the individual rat as the effect of the doses of CCl_4 accumulate. The problem with respect to the mechanism of the development of cirrhosis was clearly stated as long ago as 1936 by Cameron and Karunaratne, who stressed that the production of cirrhosis by CCl_4 in the rat depended upon inflicting repeated damage to the liver, and that each episode of damage must be confined within a narrow and critical range between a reversible "hepatitis" on the one hand and death from acute liver failure on the other. It is this need to sustain such a balancing act through many doses and over many weeks or months, with little or no information as to the condition of the liver during the process, which has stunted the development of this much needed experimental model.

We describe a new and simple approach to this old problem, in which both the variation in response to CCl_4 and the maintenance of a critical level of damage are monitored by accurately measuring the daily body weight change of the rat in response to an *intragastric* dose of CCl_4. This result is then used to calibrate the next dose of CCl_4, the weight change of which is used to calibrate the next dose, and so on until a standard level of severe decompensated cirrhosis is reached, usually after 8–10 doses. The method is equally applicable to younger (200–300 g) and older rats (450–600 g).

MATERIALS AND METHODS

Animals.—Male rats (Wistar, Charles Rivers) weighing about 150 g in the younger group and 450 g in the older group at the start of the experiment, were maintained on a stock pellet diet (Dixons FF(M)).

Procedures.—Phenobarbitone was added to the drinking water (35 mg/dl) to increase the sensitivity of the liver to CCl_4 by enzyme induction (McLean *et al*., 1969). After 10–14 days on phenobarbitone, when the younger rats were about 200–250 g and the older rats about 500 g in weight, and the stimulated liver was at its maximum size (Chatamra and Proctor, 1981), the first intragastric dose of CCl_4 was given.

Intragastric CCl_4.—Previous (unpublished) studies had convinced us that the traditional twice-weekly dosage of CCl_4 for the production of cirrhosis did not allow sufficient time for adequate recovery of the liver between doses, and that weekly doses gave better results and were probably optimal, since by day-10 post-CCl_4 recovery of the liver was virtually complete. The CCl_4 was given intragastrically after light (2 min exposure) anaesthesia with halothane/oxygen. The rats were not starved before receiving the CCl_4 as this exaggerated the response in an unpredictable manner. The cannula for the intragastric intubation was made from a fine intravenous catheter (Portex; red; 5FG; o.d. 1·65 mm) cut to 12 cm long, with the end fused into a bullet-nosed shape with a side hole. The dose was given at the same time of day (9.00 h) once a week. The need for atraumatic intubation of the oesophagus becomes of even greater importance later in the experiment when oesophageal varices and loss of clotting factors develop with the onset of cirrhosis.

Weighing.—Accurate daily weighing of the rats was carried out at the same time (9.00 h) each day using either a modified industrial electronic weighing machine (Gravitron, International Electronics) with a weight averager to dampen the activity of the rat, or more accurately when the rat was lightly anaesthetized with halothane/oxygen. Under these conditions the weight changes could be measured with an accuracy of ± 1 g in the 200–600 g range unanaesthetized or $\pm 0{\cdot}5$ g anaesthetized.

RESULTS

Calibration of doses of CCl_4

Initial dose (*mortality rate*).—The first intragastric dose of CCl_4 is particularly important in that it measures the level of response of the individual rat at the start of the experiment, and sets the pattern for subsequent doses. Once the initial dose is established for the particular type of rat/diet/conditions etc. being used, it suffices for all subsequent series provided that the

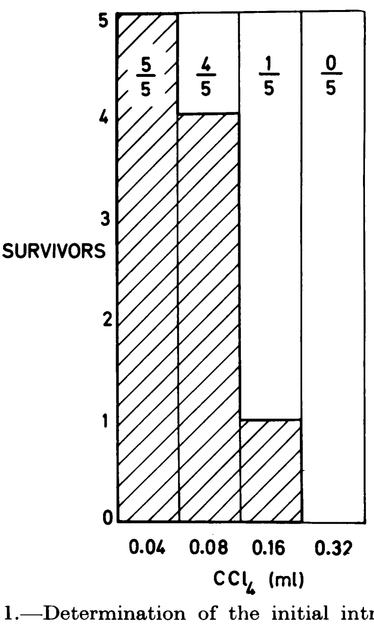


FIG. 1.—Determination of the initial intragastric dose of CCl_4 by a simple doubling dose method, using mortality and not morbidity (unlike Fig. 2). The "initial dose" is defined as that dose which is half the dose at which deaths begin to occur, and in this case is 0·04 ml. At this stage it is only an approximation of the correct dose, and the second dose (see Fig. 3) begins the process of matching the subsequent doses to the individual rats.

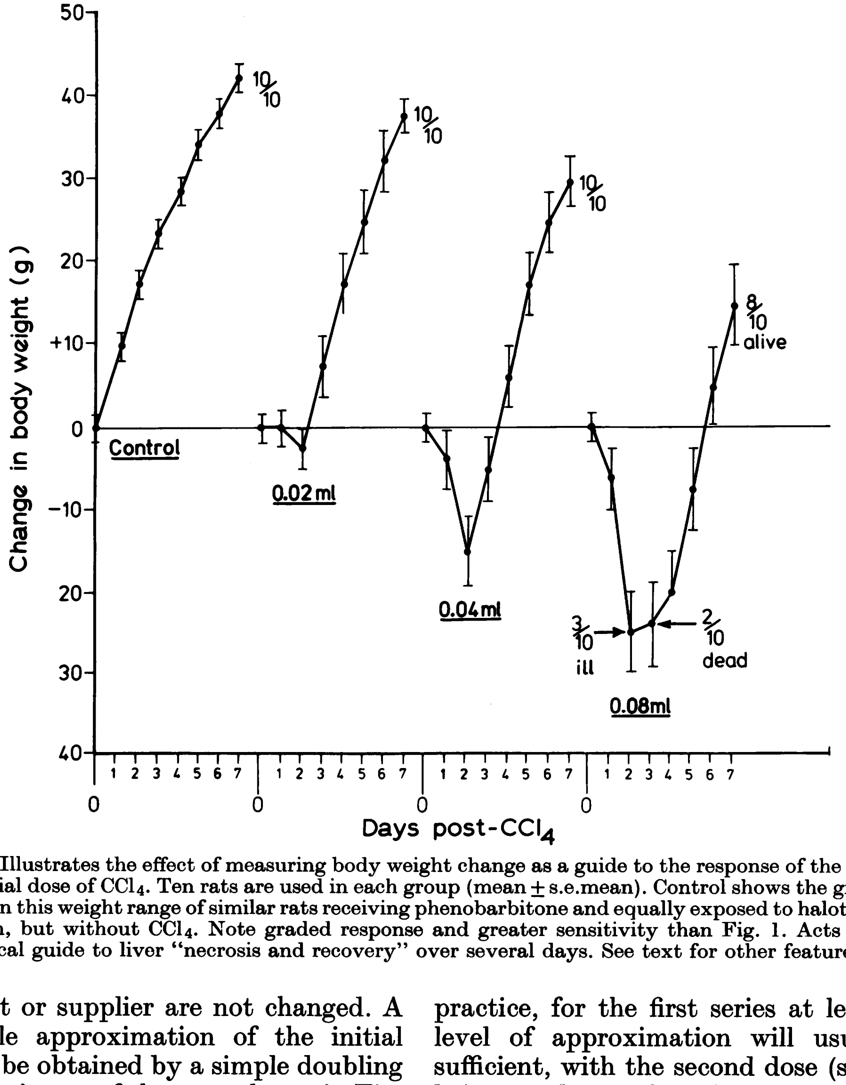


FIG. 2.—Illustrates the effect of measuring body weight change as a guide to the response of the rat to an initial dose of CCl_4. Ten rats are used in each group (mean ± s.e.mean). Control shows the growth curve in this weight range of similar rats receiving phenobarbitone and equally exposed to halothane/oxygen, but without CCl_4. Note graded response and greater sensitivity than Fig. 1. Acts as an empirical guide to liver "necrosis and recovery" over several days. See text for other features.

type, diet or supplier are not changed. A reasonable approximation of the initial dose can be obtained by a simple doubling dose experiment of the type shown in Fig. 1, where the response to a single intragastric dose is shown in terms of mortality only. In this case 20 rats are divided into 4 groups of 5, each at about 250 g body weight. The "initial dose" is defined as that dose which is half the dose at which deaths begin to occur. Note that it is a *threshold* type of dose and not an LD_{50}. The initial dose can be determined with greater accuracy by using more rats to a group or by using more doses of CCL_4 between the threshold doses, *i.e.* between in this case 0·04 ml and 0·08 ml, but in practice, for the first series at least, this level of approximation will usually be sufficient, with the second dose (see later) being used to refine the response to the first dose.

Initial dose (*body weight change*).—Fig. 2 illustrates the effect of measuring the body weight change compared with using mortality as a guide to the response of the rat to an initial dose of CCl_4. Ten rats are used in each group, and a control group indicates the growth curve in this weight range of similar rats receiving phenobarbitone and equally exposed to halothane, but without CCl_4. The advantages of the weight change measurement can clearly be seen:

(a) It is more sensitive, indicating a response to doses of CCl_4 as low as 0·02 ml and 0·04 ml, whereas the death rate at these doses is merely nil.

(b) It is a graded type of response, unlike the "either-or" of the death rate, and is a record of liver necrosis and recovery over several days rather than the single point reading of death.

(c) It indicates the point of maximum toxic response at 48–72 h, depending on the dose, acting as as a type of end-point.

(d) It can give information about the quality of survival, in that the condition of the surviving rats of the 0·02 ml group (which are virtually back on the control growth curve by day 7, immediately before the next dose) is likely to be better than the condition of the survivors of the 0.08 ml group, which are about 30 g or 10–15% short of the control weight.

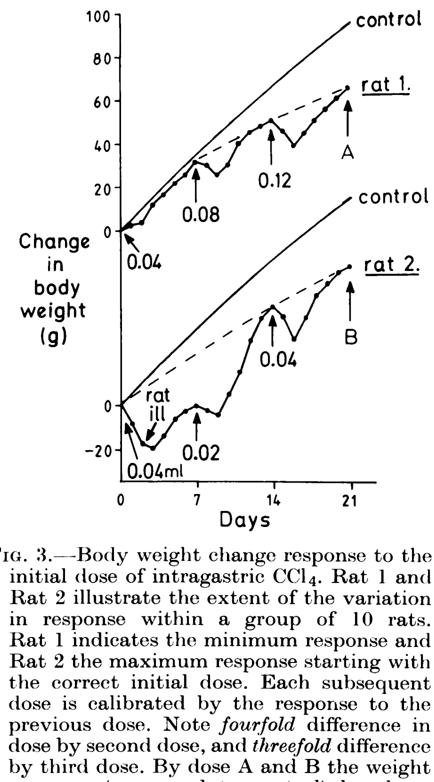


FIG. 3.—Body weight change response to the initial dose of intragastric CCl_4. Rat 1 and Rat 2 illustrate the extent of the variation in response within a group of 10 rats. Rat 1 indicates the minimum response and Rat 2 the maximum response starting with the correct initial dose. Each subsequent dose is calibrated by the response to the previous dose. Note *fourfold* difference in dose by second dose, and *threefold* difference by third dose. By dose A and B the weight response (compared to control) has been matched by changes in dose. Note sustained departure in weight (dashed line) from control weight as critical damage level is maintained. Control is mean growth curve of similar group of rats (phenobarbitone-induced/halothane-exposed) without CCl_4.

Calibration of subsequent doses of CCl_4 after the initial dose

Unlike Fig. 2, which deals with groups of rats, Fig. 3 illustrates the variation in response of individual rats to CCl_4 which can occur, as expressed by the two extremes of weight change (and morbidity) obtained with a group of 10 rats using the established initial dose (0·04 ml in our rats) of CCl_4. Rat 1 indicates the minimum response, and Rat 2 the maximum response (short of death) which can occur; the majority of results within the group lie between these two. The lines marked "control" represent the mean growth rate, without standard errors for clarity, of similar phenobarbitone-induced/halothane-exposed rats without CCl_4. In previous (unpublished) studies it was found that there was no significant difference with respect to growth, histology and standard liver function tests between groups of rats which received phenobarbitone only; halothane/oxygen only; phenobarbitone plus halothane/oxygen, and normal rats receiving none of these. Halothane, in spite of the faint possibility of hepatotoxicity, was preferred to sodium pentobarbitone for its convenience and speed of induction and recovery; and to ether because it was non-irritant and could be used with oxygen, so that rats were never hypoxic during anaesthesia, a point of particular importance in the presence of cirrhosis with ascites and hydrothorax.

It will be seen that in Rat 2 the maximum weight loss occurs by days 2 and 3 post-CCl_4; this is about 20 g absolute, but when compared with the control (*i.e.* what the weight would have been in the absence of CCl_4) the weight loss is closer to 30–35 g. For Rat 2 the second day is the point of maximum toxicity in systemic terms, with the rat being visibly ill; it does, however, recover rapidly after day 3, but though it approximates to its pre-CCl_4 weight by day 7 when the second dose is due, it is still around 35 g less than its control weight. On the other hand, the weight of Rat 1, which is minimally affected by the same dose, has virtually reached the control level by day 7. It follows, therefore, that the *second* dose of CCl_4 will be different for Rats 1 and 2.

Experience with the weight response to CCl_4 has shown that the response of Rat 1 is insufficient to initiate cirrhotic change even if continued for many months, and that it is necessary to double the dose of CCl_4 to 0·08 ml for the second dose. Conversely, experience has shown that the dose for Rat 2 is too large to be repeated, and would result in acute liver failure with the second dose; this is, therefore, reduced to 0·02 ml. It can now be seen that there is a *fourfold* difference in dose between the two extreme rats by the second dose. The other rats in the series will have proportionately different doses.

In Rat 1, the second dose of 0·08 ml causes a larger, but from our experience still inadequate weight loss; and in Rat 2 the second dose of 0·02 ml causes a smaller response, but one which is sufficient to maintain the "pressure" on the liver and yet permit the rat to equal Rat 1 by day 7 of the second dose. From experience, and having due regard to the effects of the second dose, the *third* dose of CCl_4 for Rat 1 is increased by half to 0·12 ml, and that of Rat 2 doubled to reach the original dose of 0·04 ml. This causes a weight loss by day 2 (about 6–9% in our rats) which is approximately equal in both rats, and found from experience to reflect sufficient damage to the liver to initiate and sustain the cirrhotic process with CCl_4.

At this point the difference in dose between Rat 1 and Rat 2 is now *threefold*. It can thus be seen why, traditionally, the deaths from acute liver failure tend to occur early in the series, and the failure to produce cirrhosis become evident late in a series, for Rat 2 and similar rats would have died in the first 2–3 doses, and Rat 1 and any similar rats could have been given the same dose for months without producing cirrhosis.

It might be thought at this point that the correct dose for Rat 1 and Rat 2 was now known and, with comparable doses for the other rats in the series, could be continued until cirrhosis resulted. Unfortunately, this is not so, for in addition to the variation in response to CCl_4 between the rats in a series, there now appears a variation in response *within* the individual rats with respect to time. This is due to two further factors; the increasing age of each rate, which reduces the sensitivity to CCl_4 (Cameron and Karunaratne, 1936), and even more important, the increasing damage to the liver with each dose of CCl_4, which reduces the amount of P450/CCl_4 "toxin" effect (McLean *et al.*, 1969). Both these factors act in the same direction to reduce the sensitivity of the rat liver to CCl_4, and require that the dose be increased with time. Set against this is the systemic response to the liver damage, in that if the rat does not regain sufficient weight or is ill after a dose of CCl_4, then the subsequent dose must be reduced (as in the second dose of Rat 2 in Fig. 3). With most rats the first two effects predominate, and the subsequent doses of CCl_4 are increased each time (but guided by the weight response) by an average of about 50% (see Fig. 4). Consequently, since the weight response following the third dose is that which experience has shown to be optimal for inducing cirrhosis at that stage, the *fourth* dose (A and B) would usually be increased by 50% (*i.e.* to 0·18 ml for Rat 1 and to 0·06 ml for Rat 2).

The dashed curve in Figs 3 and 4 is not selected beforehand, but is merely the result of connecting the 7-day post-CCl_4 points on the graph. It is used as a visual device to illustrate the accumulated weight loss which results from repeated cycles of weight loss and recovery, and acts as a guideline to enable the beginner in this method to anticipate the weight changes involved. Often the 7-day post-CCl_4 points will approximate to a simple curve, although minor variations in weight loss and recovery will mean that not all the 7-day points are on the curve, as indeed is the case with dose 2 (Rat 2) in Fig. 3,and dose 5 in Fig. 4. Both Figs 3 and 4, although representing the weight changes of actual rats, have been chosen to portray idealized "typical" weight change patterns which embrace most of the events which can occur to the weight of a rat during an 8–10 dose period. In practice, each rat is different and each dose of CCl_4 after the initial dose must be guided by the weight change of the previous dose, which is the essence of this report.

Typical weight change pattern for the rapid induction of micronodular cirrhosis (1–9 *doses of* CCl_4)

Fig. 4 outlines the overall pattern of weight response for the major part of a series (somewhere between the extremes of Rat 1 and Rat 2) in a younger rat, to the point where ascites appears and is sustained. In this case, for clarity, the doses are recorded in terms of the initial dose of 0·04 ml, which is taken as "X" The weight response to 0·04 ml (or "X") in Fig. 4 is judged to be adequate at this weight (230 g), and the *second* dose increased by 50% (1·5X or 0·06 ml), and so on. Note that the *fourth* dose (3X or 0·12 ml), causes an excessive weight loss, and more disturbing, a sustained weight loss on day 3, so that the subsequent *fifth* dose is reduced to 2X. This permits a recovery whilst maintaining some "pressure" on the liver, and the *sixth* dose returns to 3X, then 4X and 5X (0·2 ml) by the *eighth* dose. This eighth dose again causes an excessive degree of weight loss, sustained to day 3, and is followed during recovery by a 'spike' of rapid weight gain and loss, known from experience to be transient ascites, and designated as (?A) in Fig. 4. As a result of recognizing this transient ascites, the *ninth* is reduced, again from experience, to the minimum which at this stage will produce an effect and yet not too severe as to produce a dangerous ascitic overload. This happens to be the same dose (0·04 ml) as the initial dose (X), but by the ninth dose the liver is in a very different condition to the liver which received the initial dose. This can be seen by the rapid development of ascites, as shown in Fig. 4 as a sustained weight gain visually scaled as (A), (A+), and (A++) (see later). Since the ascites is sustained by day 7, no further doses of CCl_4 are given as it is known that the rat now has established and irreversible micronodular cirrhosis (see later).

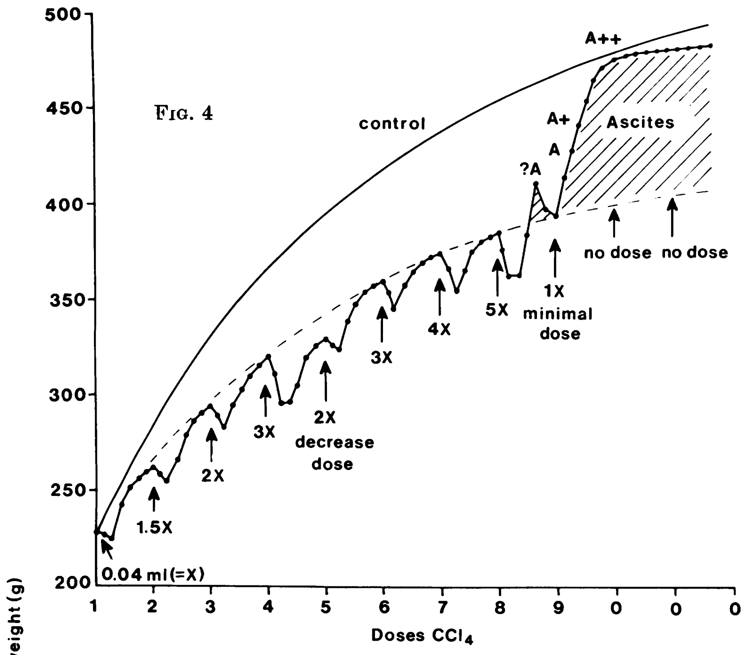
Development of cirrhosis in older rats

Fig. 5 illustrates the overall body weight changes (as mean and standard errors) of the 7-day post-CCl_4 points in 2 groups of 10 rats in which the CCl_4 is started at a mean weight of 270 g and 480 g. It is important in terms of calibrating the CCl_4 doses to note that in the younger group (which is similar to Fig. 4), the overall growth curve, although less than the control, is still a rising curve, whereas in the older rats the overall weight change (until ascites develops) is a decline. This implies that even with the correct dose each time the older rats should not be expected to regain or exceed their original weight by day 7, before the next dose is given.

Ascites

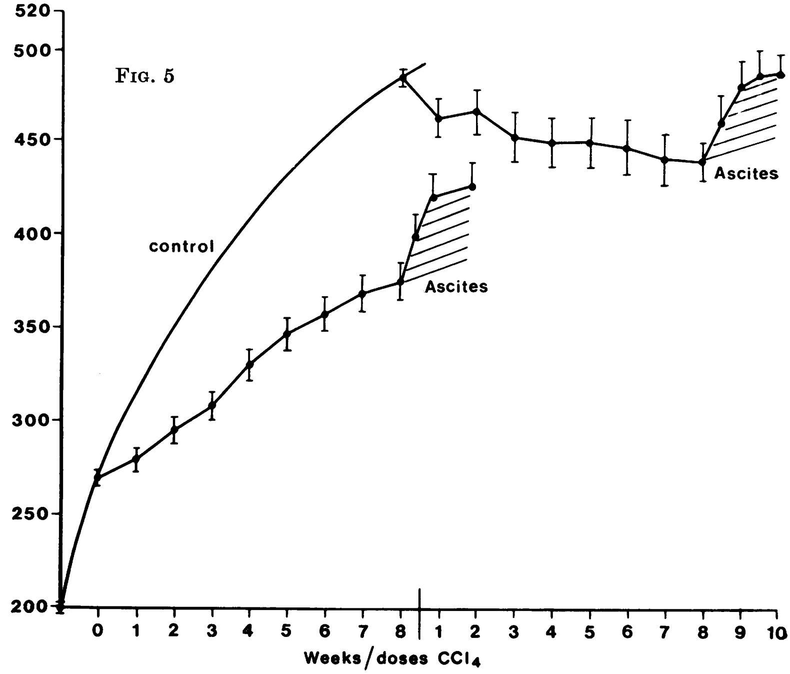
The importance of recognizing a transient "spike" type of ascites is the fact that it represents a threshold condition which necessitates a marked reduction in the dose of CCl_4. Other forms of ascitic development often occur, from the slow onset type in which minimal doses can be given to increase the ascites to the critical (A+) level, to the rapid onset type in which the volume of fluid suddenly "bolts" into an abrupt overload with hypoxia and hypercapnia from a reduction in pulmonary volume with the development of hydrothorax.

After 8–10 weekly intragastric doses of CCl_4, 72% (69 out of 96 rats) had fully developed decompensated micronodular cirrhosis with gross ascites. Under these dose-calibrated conditions it was found that micronodular cirrhosis (see Fig. 6) was always associated with at least 28 ml of ascites fluid, and that the histological



500
450
400
350
300
250
200
control
A++
A+
A
?A
Ascites
no dose
no dose
1X
minimal
dose
5X
4X
3X
2X
decrease
dose
3X
2X
1.5X
0.04 ml (=X)
1 2 3 4 5 6 7 8 9 0 0 0
Doses CCl_4
Body weight (g)

FIG. 4



520
500
450
400
350
300
250
200
control
Ascites
Ascites
0 1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8 9 10
Weeks/doses CCl_4

FIG. 5

TABLE.—*Calibration of visual assessment of ascites in anaesthetized rats*

Group	Visual assessment of ascites (ml) ?A	A	A+	A++	A+++
1	8	20	32	72 (8)	95 (10)
2	11	17	38	60 (9)	110 (12)
3	13	16	28	55	125 (14)
4	11	24	40	55	111 (10)
5	15	22	28	48	85 (9)
6	10	16	42	75 (6)	105 (10)
Mean ± s.e. mean	11·3 ± 1·0	19·2 ± 1·4	34·6 ± 2·7	60·8 ± 4·2	105·2 ± 5·5
Range	8–15 ml	16–24 ml	28–42 ml	48–75 ml	85–125 ml

Visual grading of ascites volumes in 30 rats after light halothane/oxygen anaesthesia to relax the abdominal muscles, with the rat *prone*. The grade range from (?A) when ascites is suspected; (A) when it is clear that ascites is present; to (A+++) the extreme form with hydrothorax (see Fig. 7). The actual volume of ascitic fluid measured after opening the abdomen. The numbers in brackets in columns A++ and A+++ are the volumes of hydrothorax.

picture was irreversible; this volume of fluid did not decrease (and often increased) when the CCl_4 was stopped, whereas smaller volumes of ascites than this often disappeared when the CCl_4 was stopped, and the histological picture often partially reverted towards normal.

Because of the importance, under these conditions, of this apparent threshold of ascites volume as a non-invasive indicator of the stage of cirrhosis, it was decided to check whether it was possible to estimate the volume of ascites visually. This was done by visually grading 30 ascitic rats into 5 groups by apparent volume, before opening the abdomen and measuring directly. This estimation was carried out after 2 min of halothane/oxygen induction, with the rat *prone* and the abdominal muscle relaxed with the anaesthetic; no other method was found reliable. The grades ranged from (?A) when ascites was suspected; (A) when it was clear that ascites was present; to (A+++) with extreme ascites and hydrothorax. The results are shown in the Table. Visual estimation of the grade of ascites was most reliable in the important range of A+ to A+++ which was associated with fully developed micronodular cirrhosis (see below). The critical threshold for the irreversible development of micronodular cirrhosis was the grade A+ (Fig. 7). At this volume of ascites (28–42 ml) or above, when the cirrhosis was produced under these standardized dose-calibrated conditions, the following range of pathological states were produced (mean ± s.e. mean):

(1) A histological picture of micronodular cirrhosis (Fig. 6).

(2) A shrunken and finely nodular liver

FIG. 4.—Typical weight change patterns for an individual rat from dose 1 to dose 9 of intragastric CCl_4, between the extremes of Rat 1 and Rat 2 in Fig. 3. For clarity, the doses are recorded in terms of the initial dose (0·04 ml = X) in order to indicate the broad pattern of dose change in response to weight change. Note sustained departure in weight (dashed line connecting most of the 7-day post-CCl_4 points) of cirrhotic rat from control. (?A) indicates visual grading of 8–15 ml of ascitic fluid (Table), which clears before the ninth dose causes sustained ascites of (A++) or about 48–75 ml (Table), which is associated with standard decompensated micronodular cirrhosis (see text). The control is the same as in Fig. 3.

FIG. 5.—Illustrates the body weight changes (mean ± s.e.mean) at the 7-day post-CCl_4 points in 2 groups of 10 rats in which the CCl_4 is started at a mean weight of 270 and 480 g. It is important in terms of calibrating the CCl_4 to note that in the younger group (which is similar to Fig. 4), the overall growth curve, although less than the control, is still a rising curve, whereas in the older rats the overall weight change (until ascites develops) is a decline. This implies that even with the correct dose each time the older rats should not be expected to regain or exceed their original weight by day 7 post-CCl_4 before the next dose is given. Control same as Figs 3 and 4.

(relative weight $3{\cdot}08 \pm 0{\cdot}12$; control phenobarbitone-stimulated liver $5{\cdot}30 \pm 0{\cdot}30$).

(3) Splenomegaly 3–5 times the control weight (relative weight $1{\cdot}01 \pm 0{\cdot}05$; control $0{\cdot}26 \pm 0{\cdot}02$).

(4) Portal hypertension ($20{\cdot}0 \pm 0{\cdot}8$ cm saline; control $9{\cdot}2 \pm 0{\cdot}3$ cm).

(5) Plasma albumin of less than 2·0 g/dl ($1{\cdot}73 \pm 0{\cdot}20$; control $3{\cdot}14 \pm 0{\cdot}08$).

(6) Testicular atrophy of at least two thirds of control weight by 8–10 weeks, and almost half of control weight by 12–13 weeks ($0{\cdot}55 \pm 0{\cdot}04$ relative weight; control $0{\cdot}98 \pm 0{\cdot}04$).

With the exception of the grosser testicular atrophy, which cannot be hurried, all these features are present after 8–10 weekly doses of CCl_4.

DISCUSSION

One of the main objectives of experimental studies is to provide models of human disease unclouded by the many variables attached to clinical diagnosis and treatment. If these models are not sharply defined pathological entities it much reduces their value when metabolic and therapeutic studies are made, particularly when the results are to be compared with those of other groups. This is even more the case with experimental cirrhosis of the liver, where the hepatotoxin used is not that causing most of the clinical disease.

The main problem in producing a consistent and predictable yield of a standardised cirrhosis with CCl_4 in the rat is the variation in response to the toxin. This can result in up to a fourfold difference in dose for the same effect on rats of the same age and weight (Fig. 3). As a consequence of this a dose which will have no lasting effect on the liver of 1 rat, no matter how long it is given for, can kill another apparently identical rat within 2–3 days. This renders the optimal conditions for the production of cirrhosis in the rat (Cameron and Karunaratne, 1936), virtually impossible without recourse to some means of monitoring the changes in the liver due to CCl_4, and modifying the doses accordingly. However, all the usual methods of assessing the liver damage (serial blood sampling, biopsy *etc.*), suffer from the problem that in the development of rapidly changing liver damage they must be repeated often, will show little of value in the early milder forms of damage (due to the rapid recovery towards normal soon after each dose), and would certainly exsanguinate the rat if carried out in the later more severe stages of cirrhosis, due to the clotting deficiency. Palpation of the spleen and urine tests, although useful, are inadequate in the absence of other corroborative tests.

We did not find it possible to eliminate the variation, even when using inbred rats of 20 generation brother/sister mating, so that some means had to be found of recognizing it in each rat without recourse to invasive procedures. Moreover, since most deaths in a CCl_4 series tend to occur early rather than late, the variation had to be known from the start of a series. The ideal system of monitoring in this situation should be one suitable for large numbers of rats, rapidly carried out, cheap, atraumatic, non-invasive, and since the liver changes are taking place relatively quickly, should be capable of being repeated on at least a daily basis.

The body weight response method fitted all these requirements. It had been known for some time that the rats tended to lose weight for a period following the administration of CCl_4, and accurate daily weighing merely quantified this fact. With experience, certain patterns of weight response were found to be associated with different aspects of liver damage in a predictable way, and so the method developed.

The traditional s.c. route of administering CCl_4 is a very slow and unreliable method of producing cirrhosis, and has been largely replaced by an even older method, inhalation of CCl_4, resurrected by the addition of phenobarbitone as an enzyme inducer by McLean *et al.* (1969). However, the inhalation method suffers

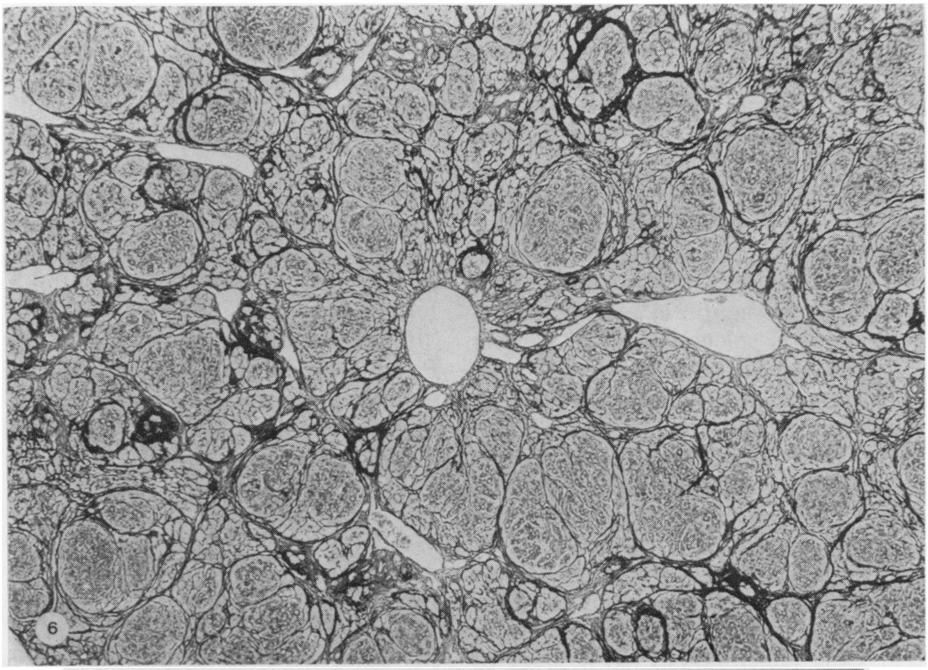


FIG. 6.—Typical picture of the histology associated with standard decompensated cirrhosis. Micronodular cirrhosis with characteristic fibrosis, clearly defined regular nodules, at 8–10 intragastric doses of CCl_4. Macroscopically, the liver is shrunken (in this case the relative weight is 2·7 g/100 g body wt compared to control phenobarbitone-stimulated liver of $5{\cdot}30 \pm 0{\cdot}30$), and finely nodular. Reticulin. $\times 20$.

FIG. 7.—Lightly anaesthetized (halothane/oxygen) prone rats with relaxed abdominal musculature, illustrating the A+ and A+ + + grades of ascites compared with a normal rat. A+ is the critical threshold volume which is associated with irreversible micronodular cirrhosis. A+ + + is invariably associated with hydrothorax.

from the fact that each dose of CCl_4 must be compressed into a period of 5–10 min, with a consequent high peak concentration of CCl_4 in the blood. Moreover, since the CCl_4 passes directly from the lungs into the left atrium, it is essentially an *arterial* dose method, and the high arterial concentrations are much more likely to produce extra-hepatic effects before the drug is sufficiently extracted and concentrated in the liver to cause the direct hepatic effect. Since the rat liver selectively concentrates CCl_4 in a ratio of 13:1 with respect to the blood (Recknagel and Litteria, 1960), it is surely better practice to adminster CCl_4 in such a way that the main part of it goes to the liver via the portal vein before entering the arterial system, thus minimizing the extra-hepatic effects. This happens when CCl_4 is given intragastrically, when it reaches a maximum level in the liver after 1·5 h, following which the concentration in the liver falls continuously to a level of about 10% of this after 20 h (Recknagel and Litteria, 1960).

Combining the intragastric route with the body weight response to CCl_4 has given us a means of empirically calibrating each dose in such a way as to maintain the optimal level of repeated damage to the liver necessary to produce cirrhosis with the minimum of extra-hepatic effects. In addition, the use of ascitic volume estimated with the rat lightly anaesthetized with halothane/oxygen has enabled the end-point of irreversible micronodular cirrhosis to be reliably determined without invasive investigations, leaving the rat in an uncompromised state for subsequent studies. Using these methods the yield of micronodular cirrhosis to date is 72% (69 out of 96 rats) of a consecutive series with 8–10 intragastric doses of CCl_4. The results are substantially the same with older rats, a model which may have more relevance clinically, although the overall growth curve tends to fall instead of rising more slowly than the control (Fig. 5), as is the case with the younger rats.

It may seem odd, with so much variation in hepatic response involved, that the end-point of micronodular cirrhosis can be predicted by a threshold level of ascitic volume, but this parallels the histology, which is itself so variable in the early stages, but of a standard pattern by 8–10 doses when the variation has been selectively "ironed-out" by repeated dose-calibration.

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