Acta vet. scand. 1969, *10*, 168—180.

From the Department of Medicine II, Royal Veterinary College, Stockholm, Sweden.

THE METABOLIC RESPONSE TO NOREPINEPHRINE IN CARBON TETRACHLORIDE POISONED SHEEP

By

J. Luthman and *G. Jonson*

In the study of liver necrosis and liver regeneration carbon tetrachloride has been widely used. The mechanisms underlying the toxic effects of carbon tetrachloride, however, have until recent years been poorly understood. The accumulation of triglycerides in the liver is one of the early signs of carbon tetrachloride poisoning. According to the modern view on fat transport, the accumulation of fat in the liver can be explained in two different ways. When non-esterified fatty acids (NEFA) are mobilized in excess from the adipose tissues, fat infiltration occurs in the liver within a few hours as was described by *Feigelson et al.* (1961). The NEFA fraction, not metabolized in the liver, is esterified and transported from the liver to the blood in the very low density lipoprotein fraction. If the synthesis of the protein moiety of the triglyceride transporting lipoproteins is depressed, the triglycerides cannot leave the liver but is stored. *Heimberg et al.* (1965) observed a decreased outward transport of triglycerides from isolated rat livers when carbon tetrachloride was added to the perfusion medium, and *Lombardi & Ugazio* (1965) showed a decrease in the very low density lipoproteins in carbon tetrachloride poisoned rats. It was further shown by *Hyams et al.* (1967) that the protein synthesis in the liver was depressed after administration of hepatotoxic agents such as

carbon tetrachloride, ethionine and azaserine. In a comprehensive review *Recknagel* (1967) gave a detailed discussion on this topic.

The mobilization of NEFA during carbon tetrachloride poisoning was discussed by *Calvert & Brody* (1960). According to their hypothesis carbon tetrachloride causes a stimulation of the adrenals with a release of catecholamines into the blood. The catecholamines are potent NEFA mobilizers and were considered to cause the fatty infiltration in the liver. Increased plasma levels of NEFA in carbon tetrachloride treated rats were reported by *Maximchuk & Rubinstein* (1963), while *Schotz & Recknagel* (1960) stated that the release of NEFA from adipose tissue did not increase after carbon tetrachloride administration.

A decrease in liver glycogen after carbon tetrachloride treatment was reported by *Campbell & Kosterlitz* (1948), *Leduc & Wilson* (1958), *Kasbekar et al.* (1959), *Rubinstein* (1962) and *Dawkin* (1963).

The rise in blood glucose following the injection of catecholamines is sometimes used as a test on the glycogen contents of the liver. Such tests were performed on ketotic cows by *Shaw* (1943).

This work was undertaken in order to study the glucose and NEFA responses to the injection of norepinephrine in sheep, before and after the administration of carbon tetrachloride.

MATERIAL AND METHODS

Seven healthy sheep were used in the study. The animals were kept indoors and fed a normal ratio of hay and grain. During the experimental period the animals were kept in metabolism cages with free access to hay and water. Norepinephrine (Nor-Exadrin, Astra) was injected intravenously in 50 ml of saline at an amount of 8 μg per kg body weight. Blood was collected from a plastic cannula in a jugular vein and analyzed for glucose according to the glucose oxidase method (reagents from AB Kabi, Stockholm) and for NEFA according to *Dole* (1956). Carbon tetrachloride was injected into the rumen at an amount of 0.8 ml per kg body weight. After carbon tetrachloride administration norepinephrine was reinjected at various times, in two animals after 9 hrs., in one animal after 24 hrs. and in two animals after 48 hrs.

The serum ornithine-carbamyl transferase (OCT) activity

was used as an indicator of the degree of liver injury. The analysis was performed according to *Reichard* (1957).

One animal, serving as a control, was injected with norepinephrine before and after a fasting period of 24 hrs.

In one animal the role of hyperglycemia on the NEFA mobilizing effect of norepinephrine was studied. The animal was given 8 μg of norepinephrine per kg body weight in 50 ml of saline, later the same amount of norepinephrine was given in 50 ml of a 10 % glucose solution.

The glucose analyses were performed immediately after the experiments, while NEFA in most cases was determined on the following day. Until analyses were performed the plasma samples were stored at —18°C. Heparin was used as an anticoagulant.

RESULTS

The response to norepinephrine in the control animal is shown in Fig. 1. The starvation period was apparently too short to cause any greater reduction in liver glycogen, as the glucose response was about the same before and after starvation. The NEFA response was almost unchanged. Before the first injection the NEFA value was 0.27 meq./l and after starvation there was a rise to 0.50 meq./l.

In the animals reinjected after 9 hrs. the glucose response was greatly reduced, while the NEFA response remained unchanged. The mean changes in the two animals are summarized in Fig. 2.

After 24 hrs. (Fig. 3) the response to norepinephrine was about the same as in the previous experiment.

After 48 hrs. (Fig. 4) the glucose response was further reduced, while the NEFA response remained unchanged.

During the first 9 hrs. the appetite was not reduced, but after 24 hrs. the animals appeared depressed and refused to eat.

Blood glucose was not altered after carbon tetrachloride administration, while there was an increase in NEFA. No sample was taken earlier than after 9 hrs. Obviously a severe liver injury had developed in all animals, as serum OCT reached very high levels. Blood glucose, NEFA and serum OCT at the time of each injection of norepinephrine are summarized in Table 1.

One animal died after a week. At autopsy severe degeneration and fat infiltration of the liver and degenerative calcification of

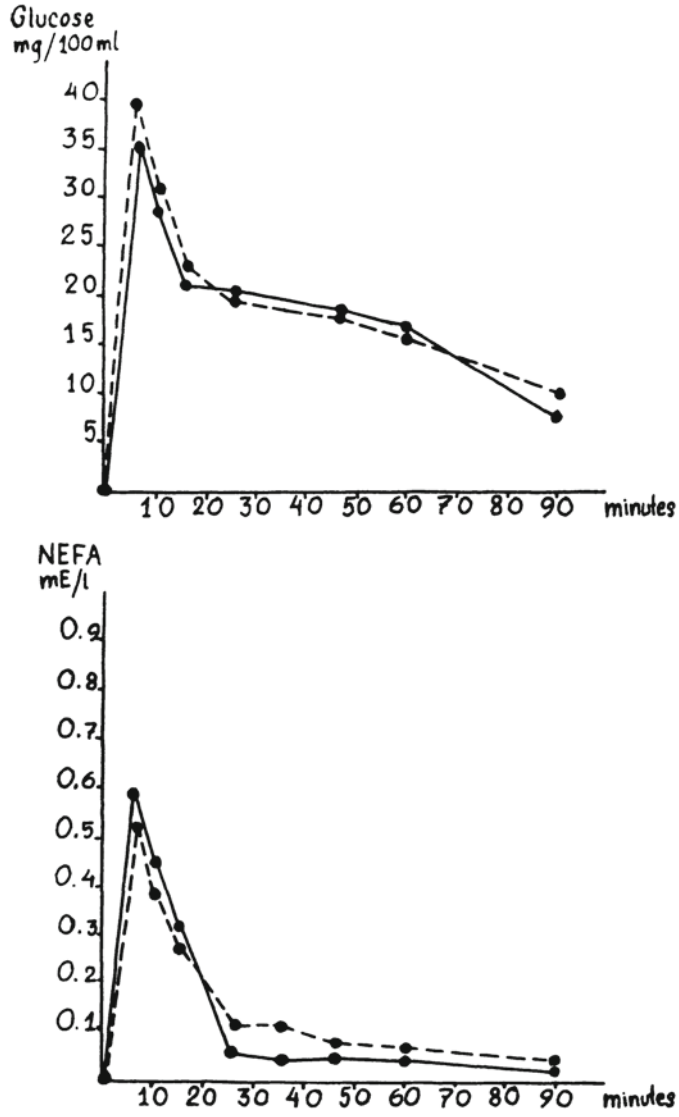

Figure 1. Changes in blood glucose and NEFA following the injection of norepinephrine in a control animal. Before ——— and after ----- a starvation period of 24 hrs.

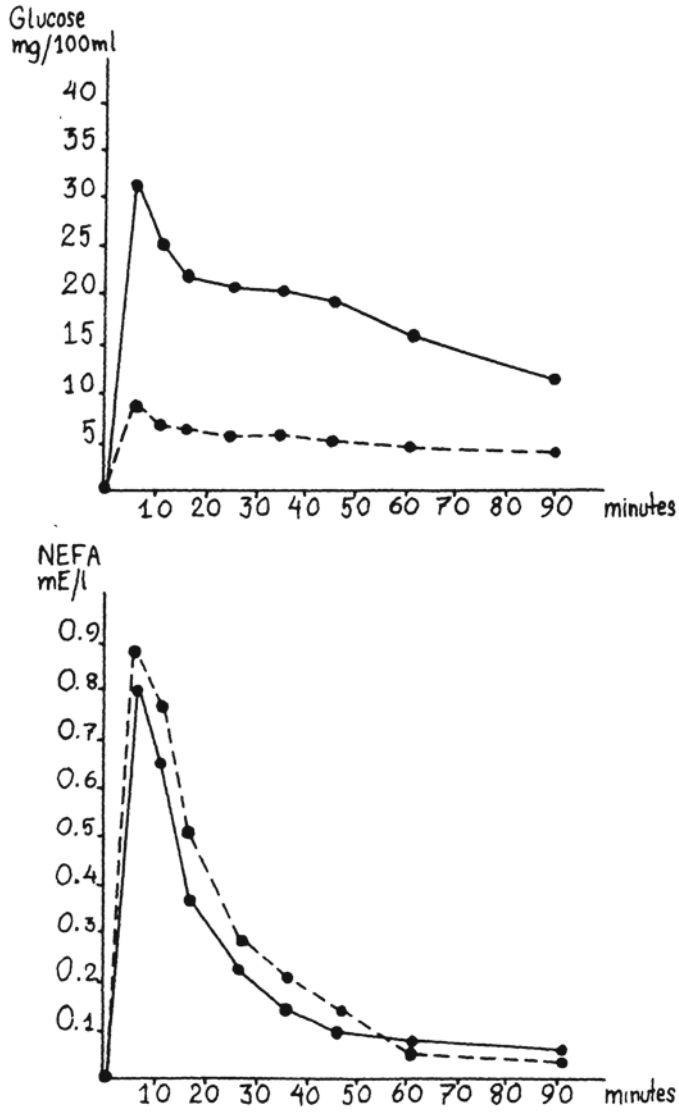

Figure 2. Changes in blood glucose and NEFA following norepinephrine, before ——— and 9 hrs. after carbon tetrachloride administration -----. Mean of two animals.

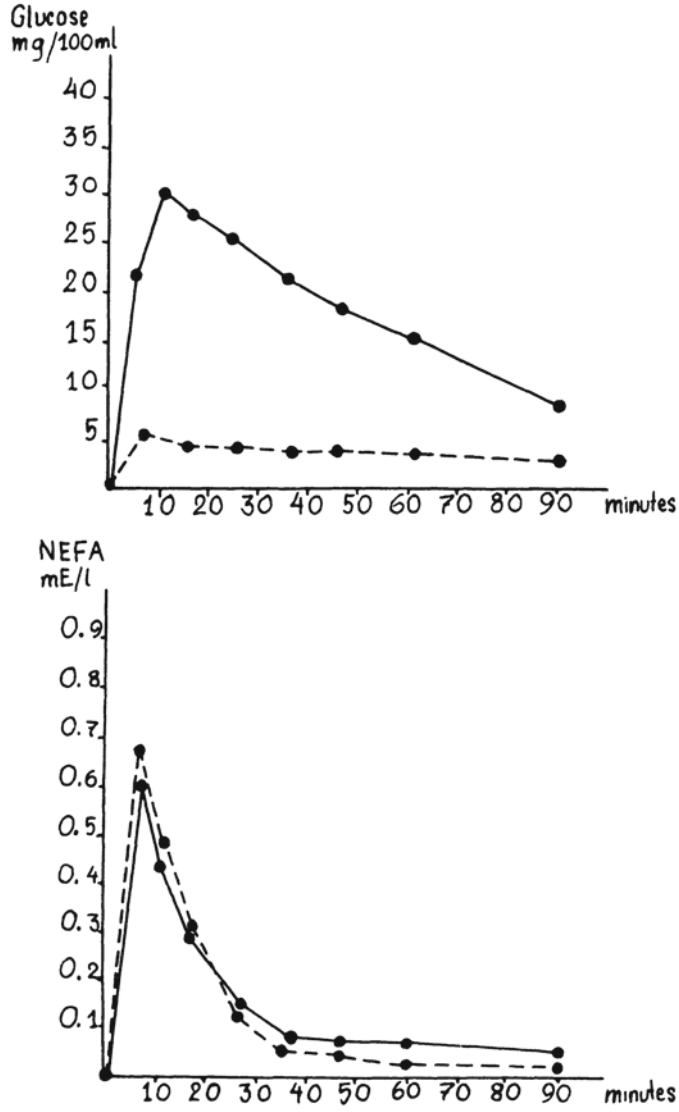

Figure 3. Changes in blood glucose and NEFA following norepineprine, before ——— and 24 hrs. after carbon tetrachloride administration - - - - - in one animal.

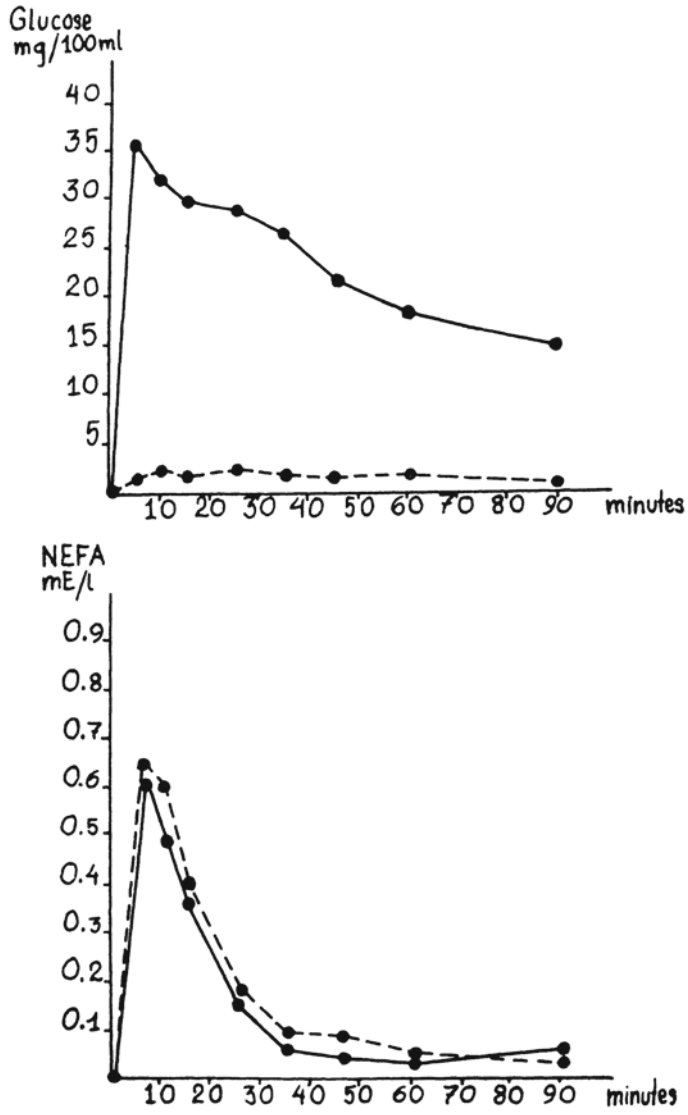

Figure 4. Changes in blood glucose and NEFA following norepinephrine, before ——— and 48 hrs. after carbon tetrachloride administration - - - - -. Mean of two animals.

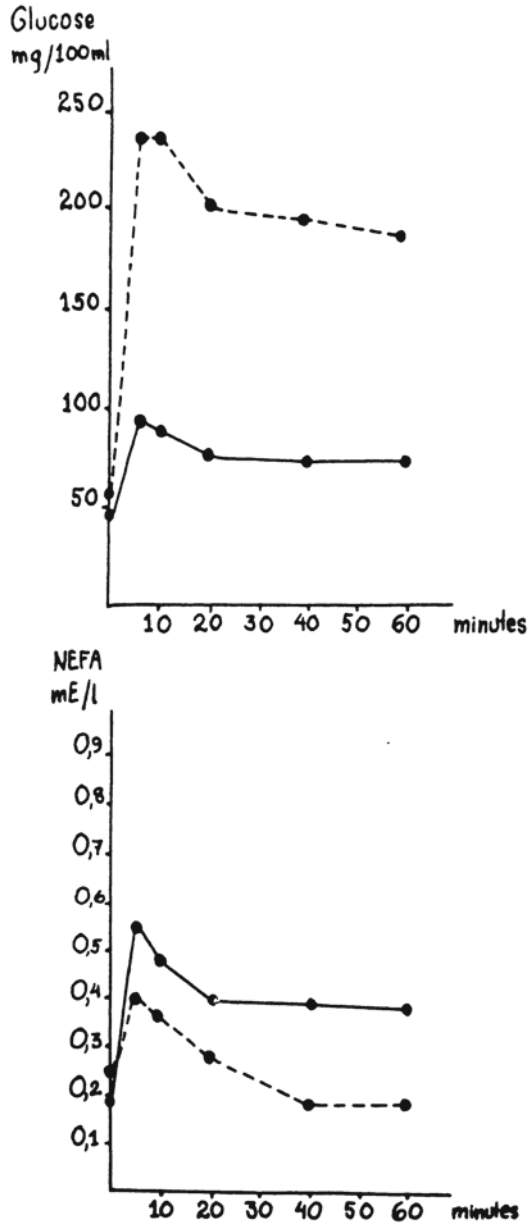

Figure 5. Changes in blood glucose and NEFA following the injection of norepinephrine in saline ———, and in a 10 % glucose solution - - - - -.

Table 1. Changes in blood glucose, NEFA and serum OCT after carbon tetrachloride administration.

Animal	Hours after carbon tetrachloride administration			
	0	9	24	48
		Glucose (mg/100 ml)		
1	38	41		
2	41	43		
3	36		33	
4	46			32
5	35			32
		NEFA (meq./l)		
1	0.18	0.31		
2	0.21	0.43		
3	0.19		0.61	
4	0.17			0.75
5	0.23			0.68
		Serum OCT (units)		
1	0.6	26		
2	1.5	32		
3	0.6		152	
4	1.5		242	150
5	1.8			180

the lungs and abomasal mucosa were noted. Another animal was killed in a very poor condition after a month. At autopsy the liver showed only a slight fat infiltration.

Fig. 5 shows the fat mobilizing effect of norepinephrine during hyperglycemia. The NEFA response was reduced when norepinephrine was given in glucose.

DISCUSSION

The dose of carbon tetrachloride used in the present study was lower than that normally used in small laboratory animals. Of the ruminants sheep are considered to tolerate carbon tetrachloride better than cattle. Deaths in cows after a dose of 50 ml was reported by *Holtenius & Jacobsson* (1966).

The reduced glucose response to norepinephrine after carbon tetrachloride administration (Figs. 2—4) indicates a reduction of the glycogen contents of the liver.

This investigation did not answer the question whether this reduction is caused by the carbon tetrachloride per se or is a

result of massive adrenal stimulation. *Rubinstein* (1962) obtained a decrease in the adrenal epinephrine level with a concomitant increase in serum epinephrine. A simultaneous increase in liver phosphorylase activity and a rise in blood glucose was observed. The blood glucose level fell to normal after 4 hrs. and liver glycogen was depleted after 7 hrs. These results showed that in the early phase of carbon tetrachloride poisoning there was a stimulation of the adrenals.

The increase in the NEFA level after carbon tetrachloride administration obtained in the present study was of the same order or somewhat greater than that reported by *Maximchuk & Rubinstein* (1963). But as was discussed by *Recknagel* (1967), these plasma levels are probably too low to be the only cause of the fat infiltration in the liver. According to our own experience (unpublished) plasma levels of about 0.6—0.8 meq./l sometimes occur in ruminants without any fatty changes in the liver. It seems therefore most probable that a block in lipoprotein synthesis is the most important cause of the fatty liver in carbon tetrachloride poisoning.

Feigelson et al. (1961) compared the effect of epinephrine and norepinephrine on blood glucose and NEFA in dogs. Norepinephrine was found to be the most suitable NEFA mobilizer since it had no glycogenolytic activity and did not affect the blood glucose level. In ruminants norepinephrine has a strong glycogenolytic effect as was reported by *Radloff & Schultz* (1966) and is seen from the results in the present study. The NEFA depressant effect of glucose was described by *Dole* (1956) and *Gordon & Cherkes* (1956), and it is shown in Fig. 5 that hyperglycemia reduced the NEFA mobilizing capacity of norepinephrine. It could therefore be expected that the rise in NEFA following norepinephrine should be greater in carbon tetrachloride poisoned animals than in normal animals, as in the former there was no rise in blood glucose. As is seen in Figs. 2—4, this did not occur. This effect of hyperglycemia is probably most pronounced during continuous infusion of catecholamines.

The results obtained in the present investigation confirm earlier findings in carbon tetrachloride poisoned animals. *Leduc & Wilson* (1958) followed the liver changes in mice by histochemical methods. As the glycogen contents of the cells waned, stainable fat accumulated. The fat infiltration occurred first within the centrilobular areas and continued until the death of

the cells. In the periportal areas the stainable fat accumulated later and disappeared after 48 hrs.

Many theories have been developed to explain the liver changes induced by carbon tetrachloride. The accumulation of fat is only one sign of a disturbed cell metabolism. The role of elevated NEFA levels in the development of the fatty changes in the liver was proposed by *Calvert & Brody* (1960). Their catecholamine hypothesis, however, was not based upon determinations of NEFA after carbon tetrachloride administration. Later *Recknagel* calculated that the transport of normal amounts of NEFA to the liver is enough to increase the fat contents if the outward transport of triglycerides is decreased. Since carbon tetrachloride causes only a moderate elevation of NEFA, a disturbed lipoprotein synthesis seems to be the main factor in the development of the fatty changes in the liver.

REFERENCES

Calvert, D. N. & T. M. Brody: Role of the sympathetic nervous system in CCl_4 hepatotoxicity. Amer. J. Physiol. 1960, *198*, 669—676.

Campbell, R. M. & H. W. Kosterlitz: The effects of short-term changes in dietary protein on the response of the liver to carbon tetrachloride injury. Brit. J. exp. Path. 1948, *29*, 149—159.

Dawkin, M. J. R.: Carbon tetrachloride poisoning in the liver of the newborn rat. J. Path. Bact. 1963, *85*, 189—196.

Dole, V. P.: A relation between non-esterified fatty acid in plasma and the metabolism of glucose. J. clin. Invest. 1956, *35*, 150—154.

Feigelson, E. B., W. W. Pfaff, A. Karmen & D. Steinberg: The role of plasma free fatty acids in the development of fatty liver. J. clin. Invest. 1961, *40*, 2171—2179.

Gordon, R. S. & A. Cherkes: Unesterified fatty acid in human blood plasma. J. clin. Invest. 1956, *35*, 206—212.

Heimberg, M., I. Weinstein, G. Dishman & M. Fried: Lipoprotein lipid transport by livers from normal and CCl_4-poisoned animals. Amer. J. Physiol. 1965, *209*, 1053—1060.

Holtenius, P. & S. O. Jacobsson: Ornithine-carbamyl transferase (OCT) activity in ruminants. Cornell Vet. 1966, *56*, 187—195.

Hyams, D. E., E. B. Taft, G. D. Drummey & K. J. Isselbacher: The prevention of fatty liver by administration of adenosine triphosphate. Lab. Invest. 1967, *16*, 604—615.

Kasbekar, D. K., W. V. Lawate, D. V. Rege & A. Sreeivasan: A study of vitamin B_{12} protection in experimental liver injury to the rat by carbon tetrachloride. Biochem. J. 1959, *72*, 384—389.

Leduc, E. H. & W. J. Wilson: Injury to liver cells in carbon tetrachloride poisoning. Arch. Path. (Chicago) 1958, *65*, 147—157.

Lombardi, B. & G. Ugazio: Serum lipoprotein in rats with carbon tetrachloride-induced fatty liver. J. Lipid. Res. 1965, *6*, 498—505.

Maximchuk, A. J. & D. Rubinstein: Lipid mobilization following carbon tetrachloride administration. Canad. J. Biochem. 1963, *41*, 525—528.

Radloff, H. D. & L. H. Schultz: Hormonal regulation of plasma free fatty acid concentration in ruminants. J. Dairy Sci. 1966, *49*, 971—975.

Recknagel, R. O.: Carbon tetrachloride hepatotoxicity. Pharmacol. Rev. 1967, *19*, 145—208.

Reichard, H.: Determination of ornithine carbamyl transferase with microdiffusion technique. Scand. J. clin. Lab. Invest. 1957, *9*, 311—312.

Rubinstein, D.: Epinephrine release and liver glycogene levels after carbon tetrachloride administration. Amer. J. Physiol. 1962, *203*, 1033—1037.

Schotz, M. C. & R. O. Recknagel: Effect of carbon tetrachloride on release of free fatty acids by rat adipose tissue. Proc. Soc. exp. Biol. (N.Y.) 1960, *103*, 398—400.

Shaw, J. C.: Studies on ketosis in dairy cattle. V. The development of ketosis. J. Dairy Sci. 1943, *26*, 1079—1090.

SUMMARY

The intraruminal administration of carbon tetrachloride to healthy sheep caused a sharp rise in the serum OCT levels. The plasma concentration of non-esterified fatty acids (NEFA) increased, while blood glucose remained unaffected. The glucose response to the intravenous injection of norepinephrine was greatly reduced after carbon tetrachloride administration, indicating a depletion of the liver glycogen. The NEFA response was not altered. As the increase in NEFA caused by carbon tetrachloride was only moderate, a block in lipoproteinsynthesis is discussed as the main factor in the development of the fatty liver.

ZUSAMMENFASSUNG

Die metabolische Antwort auf Noradrenalin bei Kohlentetrakloridvergifteten Schafen.

Die intraruminale Zufuhr von Kohlentetraklorid verursachte bei Schafen eine scharfe Steigerung von Serum-OCT. Die Plasmakonzentration von nicht veresterten Fettsäuren (NEFA) stieg, weil die Konzentration von Blutzucker nicht verändert wurde. Die intravenöse Injektion von Noradrenalin verursachte keine Blutzuckersteigerung bei vergifteten Tieren, aber der Anstieg von NEFA war derselbe als bei normalen Tieren. Der Anstieg von NEFA nach Zufuhr von Kohlen-

tetraklorid ist vermutlich nicht genug eine Fettinfiltration im Leber zu verursachen. Eine Störung in der Syntese von Lipoproteinen ist vermutlich die Hauptursache der Fettinfiltration im Leber bei Kohlentetrakloridvergiftung.

SAMMANFATTNING

Metaboliska effekter av noradrenalin hos koltetrakloridförgiftade får.

Intraruminal tillförsel av koltetraklorid gav hos får en kraftig stegring av serum-OCT. Plasmakoncentrationen av icke förestrade fettsyror (NEFA) ökade, medan blodglykoskoncentrationen förblev oförändrad. Intravenös injektion av noradrenalin gav ingen blodglykosstegring hos koltetrakloridförgiftade djur. NEFA-svaret var detsamma som hos normala djur. Ökningen av NEFA efter koltetrakloridtillförseln var förmodligen för liten för att ge upphov till någon leverförfettning. En rubbning i lipoproteinsyntesen diskuteras som den huvudsakliga orsaken till leverförfettningen vid koltetrakloridförgiftning.

(Received November 25, 1968).