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THE PREVENTIVE EFFECT OF NICOTINIC ACID ON CARBON TETRACHLORIDE TOXICITY IN SHEEP

By

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The biochemical changes in the liver cells following carbon tetrachloride administration have been intensely studied. *Christie & Judah* (1954) found that the enzyme systems first affected were those requiring pyridine nucleotides for their activity. This lead *Gallagher & Simmonds* (1959) to study the protective effect of nicotinic acid on carbon tetrachloride toxicity, as nicotinic acid is a precursor to pyridine nucleotides. It was found that nicotinic acid prevented deaths in rats given large doses of carbon tetrachloride. Later it was shown that nicotinic acid also reduced the histopathological liver changes in carbon tetrachloride poisoned sheep (*Gallagher* 1960). Other agents effective in preventing the toxic effects of carbon tetrachloride are antioxidants (*Gallagher* 1961, 1962, *Kondos & McClymont* 1967).

Gallagher (1962) found that in animals treated with antioxidants the pyridine nucleotides in the liver were maintained at normal levels despite carbon tetrachloride administration. The action of antioxidants is explained by the theory advanced by *Recknagel & Goshal* (1966). According to this theory the central and primary lesion of carbon tetrachloride is a destructive lipoperoxidation of the structural membranes within the liver cells.

In a previous report (*Luthman & Jonson* 1969), fat mobilization in carbon tetrachloride poisoned sheep was discussed. As the plasma concentration of non-esterified fatty acids (NEFA) was only moderately increased, an intensified mobilization of body

fat was not considered to be the cause of an increased fat infiltration in the liver. It was further shown that liver glycogen decreased after carbon tetrachloride administration. This finding was not based on chemical analysis, but on the blood glucose response to the intravenous injection of norepinephrine. The results obtained agreed with the opinion of *Recknagel* (1967) that a disturbed triglyceride transport from the liver is the main cause of the increased fat infiltration.

Nicotinic acid is of interest not only as a precursor to pyridine nucleotides, but also because of its antilipolytic properties. *Carlson & Orö* (1962) demonstrated that nicotinic acid depressed the plasma level of NEFA and almost completely inhibited the norepinephrine induced lipolysis. *Carlson & Liljedahl* (1963) reported that nicotinic acid prevented norepinephrine induced fatty changes in dog livers. This was apparently due to the depressed plasma level of NEFA. In carbon tetrachloride poisoning the cause of the increased fat content in the liver is not an overload with fatty acids, but probably a decreased lipoproteinsynthesis. Since nicotinic acid also prevents the degenerative changes in the liver, it can be assumed that the antilipolytic properties of nicotinic acid is of only minor importance in the prevention of carbon tetrachloride toxicity.

The role of butyric acid in the intermediate metabolism of the ruminants was discussed previously (*Luthman & Jonson* 1968). It was assumed that butyric acid acts glycogenolytically when given intravenously. This assumption was based on the finding that in a ewe with pregnancy toxemia there was no blood glucose response to the intravenous injection of butyric acid.

The object of the present investigation was to study the glycogenolytic effect of butyrate in normal sheep and after glycogen depletion by carbon tetrachloride. The aim was also to study the preventive effect of nicotinic acid on carbon tetrachloride poisoning. The effect of nicotinic acid on the liver was also studied.

MATERIAL AND METHODS

The animals used were mature sheep of both sexes, weighing from 35 to 50 kg. The animals were kept in metabolism cages with free access to hay and water. The glycogen content of the liver was tested by intravenous injection of butyric acid, 2.5 mM/kg. Before use the acid was buffered with saturated NaOH to a

pH of 7.2 and diluted with saline to a volume of 50 ml. Blood was sampled at frequent intervals and blood glucose was determined according to the glucose oxidase method. Carbon tetrachloride, 0.8 ml/kg, was given intraruminally to two animals. Liver glycogen tests were performed immediately before and 24 hrs. after carbon tetrachloride administration. Another pair of animals was pretreated with nicotinic acid, 50 mg/kg, on the day before carbon tetrachloride administration. The nicotinic acid was dissolved in saline and injected directly into the rumen. Liver glycogen was tested before and 24 hrs. after carbon tetrachloride treatment. The effect of a larger dose of nicotinic acid, 100 mg/kg, given simultaneously with carbon tetrachloride was studied in two animals, and to another pair of animals only the larger dose of nicotinic acid was given. A control animal was given only butyric acid on two subsequent days.

Serum OCT, determined according to *Reichard* (1957), was used as a test of liver injury. Three sheep were used for histological examinations. Two of these animals were given carbon tetrachloride intraruminally, one of them was pretreated with 50 mg/kg of nicotinic acid on the previous day. The third sheep was given 100 mg/kg of nicotinic acid. The animals were killed after 24 hrs. and samples from the livers were taken for histological examinations. The samples were fixed in formalin and alcohol. Sections from the formalin fixed material were stained with hematoxylin and eosin and with Sudan Black, and sections from the alcohol fixed material were stained for glycogen with periodic acid Schiff stain.

RESULTS

The changes in blood glucose following the injection of butyric acid immediately before and 24 hrs. after carbon tetrachloride administration are shown in Fig. 1. After carbon tetrachloride administration the glucose response was greatly reduced. In the animals pretreated with 50 mg/kg of nicotinic acid the rise in blood glucose was about the same before and after carbon tetrachloride administration (Fig. 2). When the double dose of nicotinic acid, 100 mg/kg, and carbon tetrachloride were given simultaneously the blood glucose response was very small (Fig. 3), the glucose rise was even smaller than in the animals given only carbon tetrachloride (Fig. 1). In the animals treated only

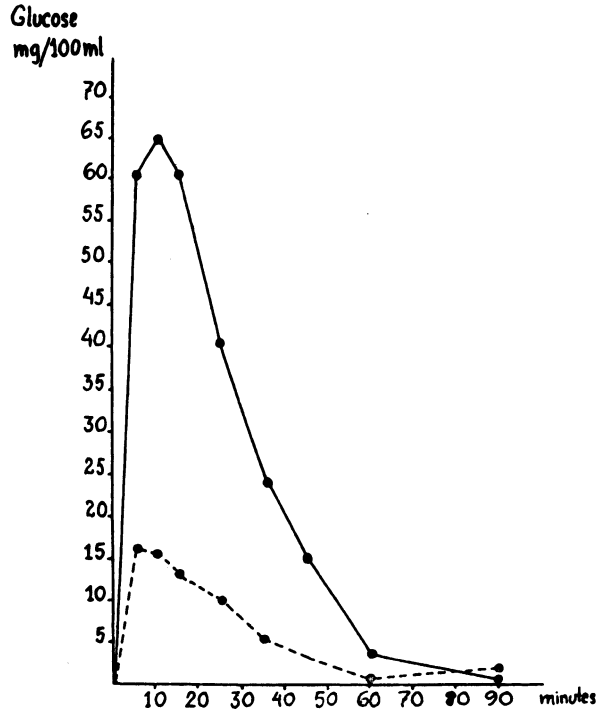

Figure 1. Changes in blood glucose following butyric acid, before ——— and after - - - - - - - carbon tetrachloride administration. Mean of two animals.

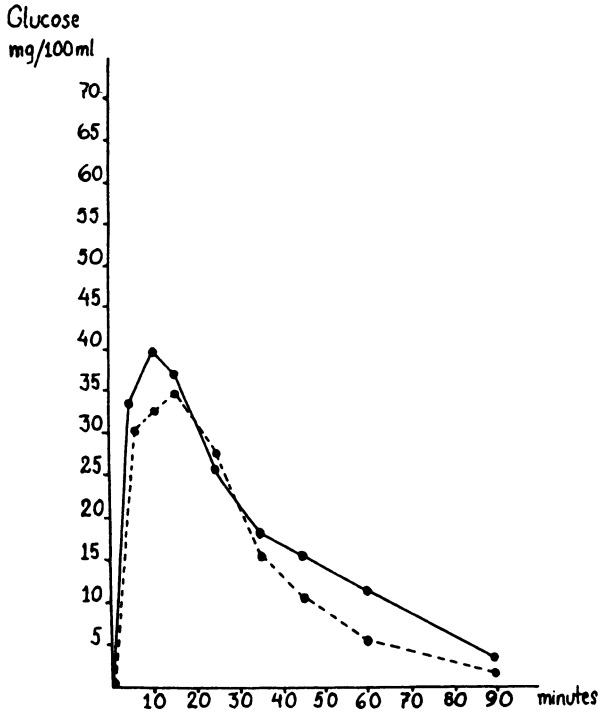

Figure 2. Changes in blood glucose following butyric acid, before ——— and after - - - - - - - carbon tetrachloride administration, animals pretreated with 50 mg/kg of nicotinic acid. Mean of two animals.

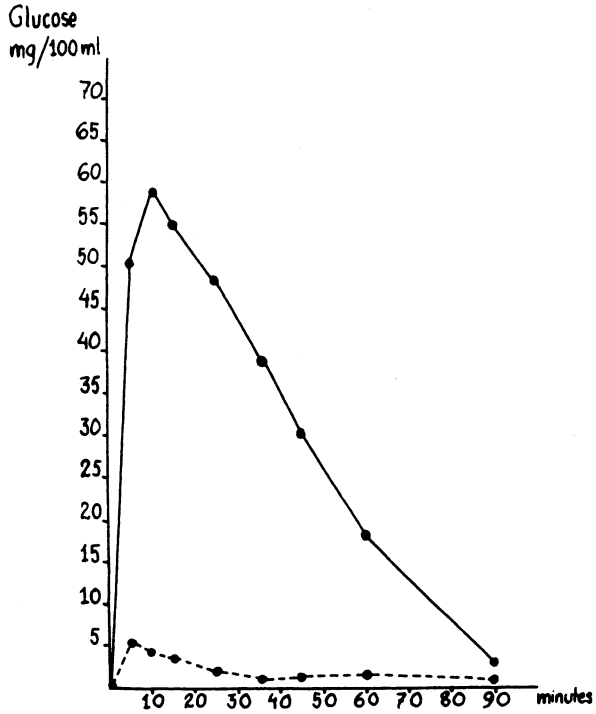

Figure 3. Changes in blood glucose following butyric acid, before ———— and after ------- simultaneous administration of carbon tetrachloride and 100 mg/kg of nicotinic acid. Mean of two animals.

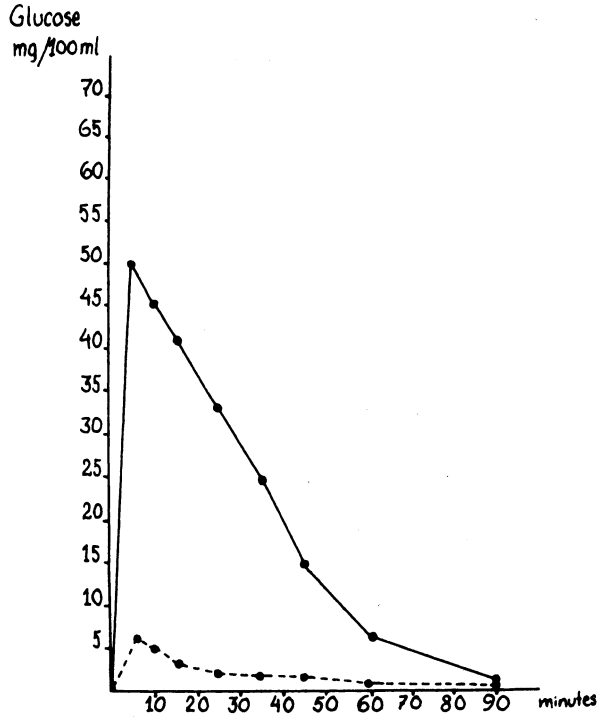

Figure 4. Changes in blood glucose following butyric acid, before ———— and after ------- administration of 100 mg/kg of nicotinic acid. Mean of two animals.

Table 1. Blood glucose (mg/100 ml) at the time of each injection of butyric acid.

Animal no.	Treatment	Day 1	Day 2
1	—	42	38
2	Carbon tetrachloride	47	39
3	„	46	41
4	Carbon tetrachloride. Pre-treatment with nicotinic acid,	44	45
5	50 mg/kg on the previous day	46	50
6	Carbon tetrachloride and nicotinic acid,	45	26
7	100 mg/kg, simultaneously	38	29
8	Nicotinic acid, 100 mg/kg	44	60
9	„	42	50

with 100 mg/kg of nicotinic acid the glucose response was reduced to about the same degree as in the animals given carbon tetrachloride and nicotinic acid simultaneously (Fig. 4). In a control animal injected with butyric acid on two subsequent days the glucose response was the same at both occasions.

One of the animals given both carbon tetrachloride and nicotine acid died after about 48 hrs. At necropsy severe edema and congestion of the lungs were noted, the heart was dilated and the liver showed marked degenerative changes.

Blood glucose at the time of the liver glycogen tests is shown in Table 1. The most striking changes occurred in the animals given the larger dose of nicotinic acid (animals 8 and 9). In both these animals there was an increase in blood glucose on day 2, in spite of reduced food intake. In animals 6 and 7, given 100 mg/kg of nicotinic acid and carbon tetrachloride simultaneously, blood glucose was reduced from 48 and 38 mg/100ml to 26 and 29 mg/100 ml respectively. In the other animals there were only minor changes. Table 2 shows serum OCT at various times. As seen from the table, serum OCT was increased in all animals, even in those pretreated with the lower dose of nicotinic acid.

At necropsy of the killed animals the only prominent changes were found in the livers. The liver from the animal given only carbon tetrachloride was very dark and showed multiple sub-

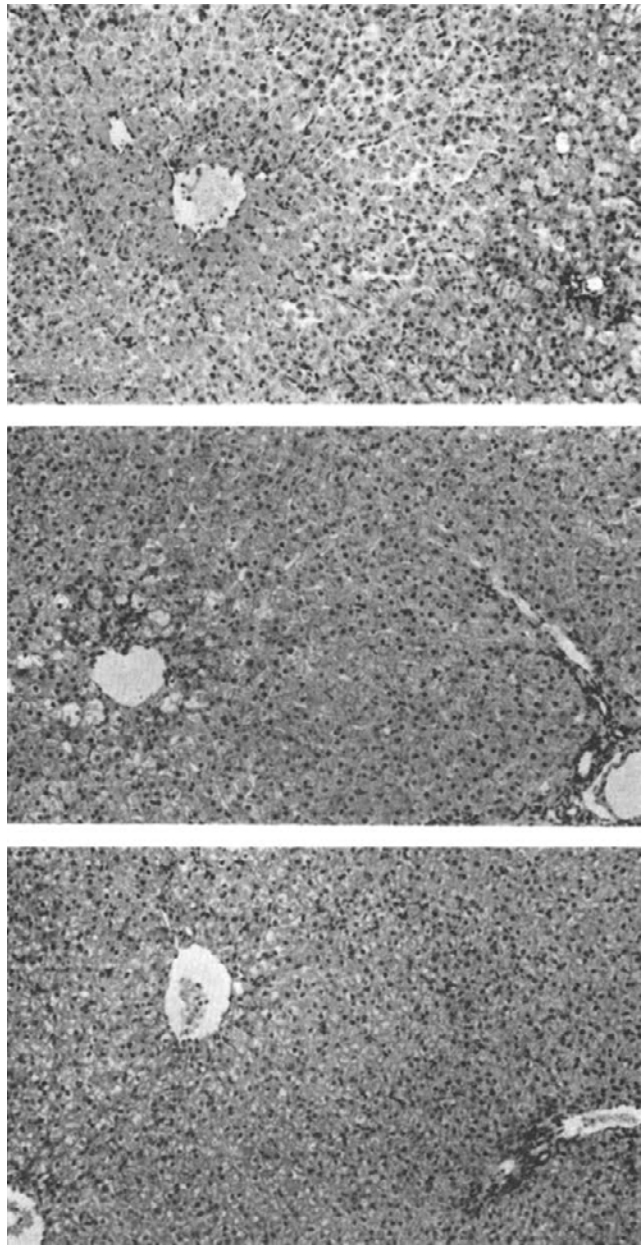

Figure 5. Livers from animals treated with carbon tetrachloride (top), carbon tetrachloride after pretreatment with 50 mg/kg of nicotinic acid (middle), and 100 mg/kg of nicotinic acid (bottom). H.E. 96 ×.

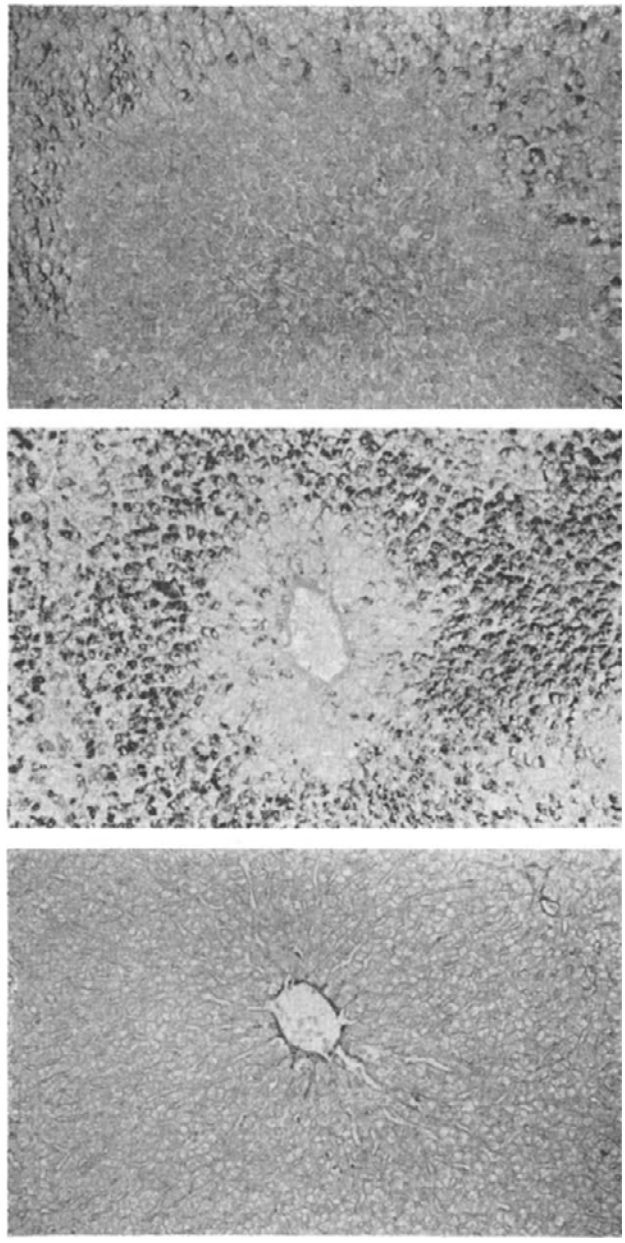

Figure 6. Glycogen content of livers from animals treated with carbon tetrachloride (top), carbon tetrachloride after pretreatment with 50 mg/kg of nicotinic acid (middle), and 100 mg/kg of nicotinic acid (bottom). PAS 96 ×.

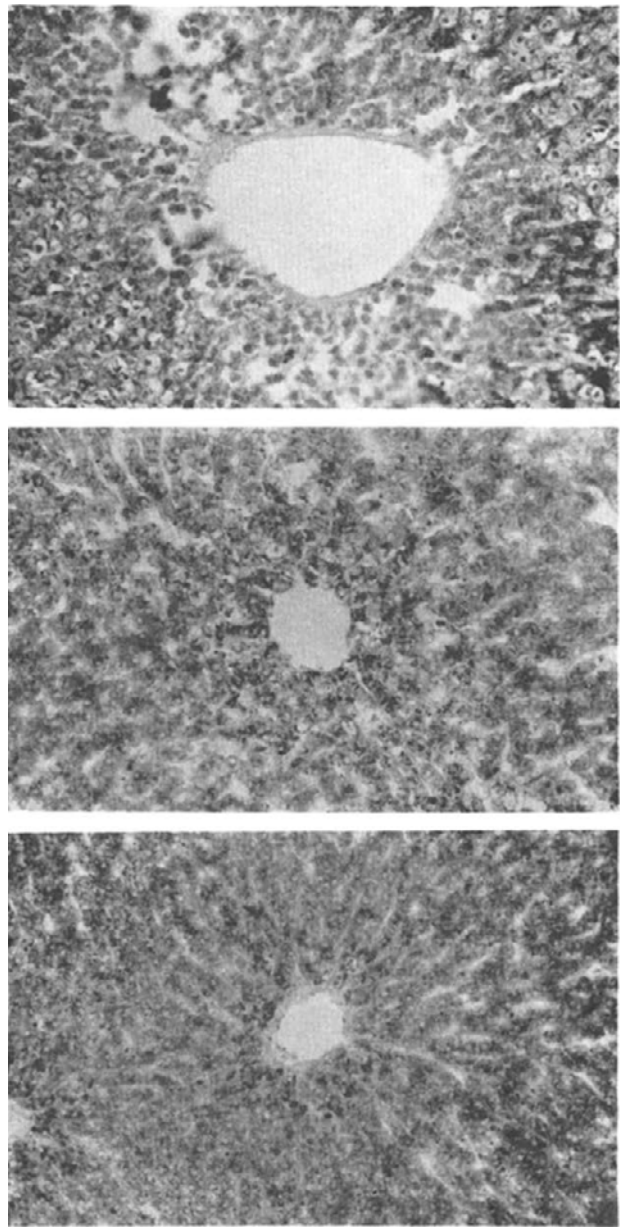

Figure 7. Fat content of livers from animals treated with carbon tetrachloride (top), carbon tetrachloride after pretreatment with 50 mg/kg of nicotinic acid (middle), and 100 mg/kg of nicotinic acid (bottom). Sudan Black 96 ×.

Table 2. Serum OCT (U) after carbon tetrachloride and nicotinic acid administration.

Animal no.	Treatment	Days				
		0	1	2	3	6
1	—	5.4	7.8	3.0	4.2	1.2
2	Carbon tetrachloride	3	93	49	23	2.4
3	„	6.6	175	114	79.5	14
4	Carbon tetrachloride*). Pretreatment with nicotinic acid, 50 mg/kg on the	5.4	171	122	50	5.4
5	previous day	1.8	128	120	72	5.4
6	Carbon tetrachloride and nicotinic acid, 100 mg/kg,	3	122	53	20.3	4.8
7	simultaneously	6.6	67**)			
8	Nicotinic acid, 100 mg/kg	9.6	15.6	39	10.8	10.2
9	„	2.4	16.7	14.4	6.6	6

*) serum OCT in animal 4 was 1.8 U and in animal 5 1.6 U at the time of nicotinic acid administration.

**) animal died shortly after sampling.

capsular hemorrhages. The animal pretreated with 50 mg/kg of nicotinic acid showed a macroscopical normal liver, while the liver from the animal given 100 mg/kg of nicotinic acid showed a slightly lighter colour than normal.

Histological examination showed that liver injuries had occurred in all animals (Figs. 5—7). The liver from the animal given only carbon tetrachloride showed severe centrolobular necrosis. Stainable fat occurred, like glycogen, only in the perilobular areas. Pretreatment with nicotinic acid reduced the necrotic changes and protected from glycogen depletion. A moderate fat infiltration occurred mainly perilobularly. At a dose of 100 mg/kg nicotinic acid caused a diffuse liver degeneration. The cells showed increased eosinophilia and pycnotic nuclei. The glycogen was completely depleted and fat containing cells were visible in the perilobular areas.

DISCUSSION

The injection of 2.5 mM/kg of butyric acid caused a rapid increase in blood glucose in all untreated animals. The blood glucose response was of the same order or even greater than observed previously after administration of 8 μg/kg of norepinephrine (*Luthman & Jonson* 1968). There was a good agreement between the glucose response following butyric acid and the glycogen content of the livers from animals treated in the same way (Fig. 6). It thus seems clear that butyric acid has glycogenolytic properties and may replace norepinephrine in liver glycogen tests in ruminants.

Pretreatment with 50 mg/kg of nicotinic acid reduced the carbon tetrachloride induced liver changes. But at the double dose, 100 mg/kg, nicotinic acid showed an exerted hepatotoxic effect and caused a moderate fat infiltration. *Carlson & Liljedahl* (1963) prevented noreprinephrine induced fatty changes in dog livers by injecting nicotinic acid at doses of 150—200 mg/kg. The increased fat content of the sheep liver following nicotinic acid may be explained in two different ways. Like other hepatotoxic agents nicotinic acid may depress lipoproteinsynthesis in the liver. The transport of fat from the liver to the blood is disturbed and fat accumulates. The fat infiltration may also be caused by an excessive mobilization of NEFA. Nicotinic acid lowers the plasma level of NEFA, but the depression is sometimes followed by a rebound far above zero level. This phenomenon was described by *Carlson & Orö* (1962) and was further studied by *Pereira* (1967).

In the experiments of *Carlson & Liljedahl* the liver fat was analyzed after an 8 hrs. norepinephrine infusion and NEFA could be kept at low levels by nicotinic acid only during 6 hrs., then NEFA increased. It seems possible that the experiments were ended at the onset of the rebound effect. In the present study lower doses of nicotinic acid were used, but samples for histological examination were taken after 24 hrs. It may also be possible that sheep are more susceptible to the toxic effects of nicotinic acid than dogs.

In man large doses of nicotinic acid, 1.5—6 g daily, was sometimes used in the treatment of hypercholesterolemia and related disorders, but hepatic complications were rare and severe histological changes did not occur (*Baggenstoss et al.* 1967).

The degenerative changes were most pronounced in the carbon tetrachloride treated animals, while 100 mg/kg of nicotinic acid

caused the most complete glycogen depletion. Despite reduced histopathological changes the animals pretreated with nicotinic acid showed very high serum OCT levels.

The decreased blood glucose levels in animals 6 and 7 on day 2 (Table 1) were probably due to a rapid break down of liver glycogen and a reduced food intake. Animals 8 and 9 showed an increase in blood glucose on day 2. According to *Baggenstoss et al.* hyperglycemia is one of the side effects reported during nicotinic acid therapy. The mild hyperglycemia in the sheep may have been caused by an increased gluconeogenesis, since the glycogen test showed a highly reduced liver glycogen content. *Kanics & Rubinstein* (1968) showed that carbon tetrachloride poisoned animals were unable to resynthetize liver glycogen and it seems possible that animals surviving acute experiments have a decreased liver glycogen content for a long time. *Kondos & McClymont* (1967) used sodium selenite and tocopherol acetate to prevent carbon tetrachloride toxicity in sheep. The results were principally the same as in the present study. Large doses of selenite and tocopherol potentiated the toxic effects of carbon tetrachloride, while smaller doses decreased the susceptibility of the sheep.

It was hoped that nicotinic acid could be used in the treatment of bovine ketosis. In ketosis the NEFA level is raised (*Adler et al.* 1963) and high NEFA levels are of importance for the development of fatty livers. Because of its hepatotoxic effects it seems however not adviceable to use nicotinic acid in doses which effectively lowers NEFA, since in ketotic cows decreased liver glycogen and slightly depressed hepatic functions are common.

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SUMMARY

The blood glucose response following the intravenous injection of butyric acid, 2.5 mM/kg, was studied in normal and carbon tetrachloride poisoned sheep. In normal sheep there was a rapid increase in blood glucose. Carbon tetrachloride greatly reduced the glucose response. In animals pretreated with nicotinic acid, 50 mg/kg, on the day before carbon tetrachloride administration, the glucose response was not altered. When a larger dose of nicotinic acid, 100 mg/kg, was given simultaneously with carbon tetrachloride, the glucose response was reduced more than after only carbon tetrachloride. Nicotinic acid alone at the dose of 100 mg/kg reduced the glucose rise somewhat more than carbon tetrachloride. There was a good agreement between

the glucose rise following butyric acid and the glycogen content of the liver. It thus seemed clear that butyric acid has a glycogenolytic effect when given intravenously. Carbon tetrachloride caused severe necrosis and fatty changes in the liver. These pathological changes were reduced by pretreating the animals with 50 mg/kg of nicotinic acid. The larger dose of nicotinic acid, 100 mg/kg, caused a diffuse degeneration of the liver cells and a complete disappearence of glycogen. All liver injuries were followed by a rise in serum OCT.

SAMMANFATTNING

Den preventiva effekten av nikotinsyra vid koltetrakloridförgiftning hos får.

Blodglykosstegringen efter intravenös injektion av smörsyra, 2,5 mM/kg, studerades hos normala och koltetrakloridförgiftade får. Hos normala får följdes smörsyrainjektionen av en snabb blodglykosstegring. Efter intraruminal tillförsel av koltetraklorid var glykossvaret avsevärt reducerat. Om djuren behandlades med nikotinsyra, 50 mg/kg, ett dygn före koltetrakloridtillförseln, påverkades inte den smörsyrainducerade hyperglykämien. När en större dos nikotinsyra, 100 mg/kg, gavs samtidigt som koltetraklorid, var glykosstegringen efter smörsyrainjektionen mycket liten. Resultatet blev detsamma om djuren behandlades med enbart nikotinsyra i en dos av 100 mg/kg. Glykogeninnehållet i levern från djur som behandlats med koltetraklorid och nikotinsyra, visade god överensstämmelse med blodglykosstegringen efter smörsyrainjektion. Det synes därför klart att smörsyra verkar glykogenolytiskt vid intravenös tillförsel. Koltetraklorid gav upphov till utbredda nekrotiska förändringar och en ökad fettinlagring i levern. Premedicinering med nikotinsyra, 50 mg/kg, reducerade dessa förändringar. I en större dos, 100 mg/kg, orsakade nikotinsyra en diffus levercellsdegeneration och en fullständig uttömning av glykogen. Alla leverskador orsakade en höjning av serum OCT.

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