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STUDIES ON THE ACTIVITY OF SORBITE DEHYDROGENASE IN NORMAL SWINE BLOOD SERA AND TISSUES AND IN SERA AFTER EXPERIMENTAL CARBON TETRACHLORIDE POISONING[1])

By

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Sorbite dehydrogenase (SDH) catalyses the reaction fructose $\overset{+\,H}{\underset{-\,H}{\rightleftharpoons}}$ sorbite (3). Diphosphopyridine nucleotide (DPN) acts as a co-enzyme.

SDH is elevated in blood-serum of human beings suffering from liver diseases (2,6, 7). Normal sera contain no enzyme or only traces thereof. *Gerlach* (6) sets the normal upper limit at 0.02 μ mol./ml./hr. ($\simeq$ 0.33 μ mol./min./litre), which is just above what can be determined with the method used. In tissues of man SDH seems to occur mainly in the liver and kidneys. A similar distribution was found in rats (1). It was present in livers from cats, mice, guinea-pigs, albino and wild rabbits, and frogs (1).

We have found no reports concerning the occurrence and distribution of SDH in swine.

MATERIAL AND METHODS

The pigs were healthy animals belonging to the Swedish Land Breed (lantras). Both females and castrated males were used.

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Preparation of tissue extracts

Tissues were collected from slaughter pigs (body-weight about 90 kg.) within 10 minutes after they had been electrically executed at the slaughterhouse. The tissue pieces were kept in ice at 0° C during the transport to the laboratory and during the subsequent manipulations, which started within a further 60 minutes. Tissue taken in the double weight amount of 0.05 M triethanol amine/HCl buffer at pH 7.6, in Ringer's solution, was homogenized at 20.000 r.p.m. for 2—3 minutes in an Ultra-Turrax homogenizer. The suspension was centrifuged at 3.000 r.p.m. for 15 minutes. The supernatant was taken for enzyme determination. For preparation of liver suspension for intravenous infusion, triethanol amine buffer was substituted for phosphate buffer saline (4) in the ratio 1:3. *Sera* were prepared from blood specimens collected from the anterior vena cava.

Enzyme determination

Glutamic oxaloacetic transaminase (GOT) was determined by *Reitman & Frankel*'s technique and ornithine carbamyle transferase (OCT) by *Reichard's* techniques as described previously (13).

SDH was determined by an optical method (2,8). 0.5 ml. of serum or supernatant was added to a mixture of 3 ml. of 0.05 M triethanol amine/HCl buffer (pH 7.6) and 0.1 ml. of $DPNH_2$ solution (100 mg. of $DPNH_2$ disodium salt, Sigma + 10 ml. of re-distilled water, pH adjusted to 7.6 by addition of $NaHCO_3$). The mixture was left in a water-bath at 25° C for 30 minutes. 0.2 ml. 40 per cent d-fructose was then added. Change of optical density per minute was measured at 340 mμ and 25° C in a thermostatically regulated 1-cm. cell (Unicam SP 500).

A change of optical density by 0.001 per minute corresponds to 0.6 international units (μ mol./min./litre).

1-phospho-fructaldolase (F-1-P-aldolase) was determined in a similar way (9, 12). After incubation at 25° C for 30 minutes as above, 0.2 ml. of fructose-1-phosphate solution (0.9 g. of fructose-phosphate-Ba-salt, "Boehringer" is dissolved in 6 ml. of re-distilled water. An equivalent amount of Na_2SO_4 solution is added. After centrifuging and washing with re-distilled water the volume is made up to 10 ml.), and 0.05 ml. of α-glycerophosphate dehydrase ("Baranowsky Ferment", Kristallsuspension "Boehringer", dilution 1:1000) is added.

The mixture is thoroughly shaken. Change of optical density is measured as above.

The standard error of a single determination of SDH, calculated from duplicate determinations on 13 sera varying from 1.1 to 81.0 units, was ± 2.45 per cent of the mean.

Experimental liver injury was produced in pigs, weighing 30—50 kg., by giving carbon tetrachloride, 0.25 ml. per kg. body-weight, into the stomach through an oesophageal tube. To avoid struggling and vomiting the pigs were pretreated with an ace-promazine preparation (Plegicil, Pharmacia, 0.5 mg. per kg. body-weight intramuscularly).

RESULTS

Serum-SDH for normal pigs (n = 42), body-weights 15—40 kg., showed a mean value of 0.44 units with s. d. ± 0.26. For comparison, GOT was also determined in the same sera. The mean value was 42.

The SDH activity of different tissue preparations from pigs is shown in Table 1.

The F-1-P-aldolase activity of the liver and kidney is shown in Table 2.

The rate of elimination of SDH, GOT and OCT after intravenous infusion of liver extract is shown in Fig. 1. When plotted on semilogarithmic paper (enzyme activity on logarithmic scale) the curves turn out to be straight lines. The interval from zero time, obtained by extrapolation, to the point indicating 50 per cent decrease of activity is shown in Table 3.

Table 1. SDH activity units of swine tissues. The homogenized tissue was diluted 1:500 (liver), 1:200 (kidney), and 1:20 (other tissues).

Pig no.	1	2	3	4	5
Liver	8670	9360	9750	6540	13050
Kidney	2400	1760	5270	3360	5480
Intestinal wall	220	206	263	162	272
Spleen	50	71	50	108	72
Lung	13	26	37	44	57
Skeletal muscle	29	33	35	24	36
Heart muscle	0	0	0	0	0
Pancreas	0	0	0	0	0

Table 2. 1-phospo-fructaldolase activity (units) of swine liver and kidney. Dilution of homogenized tissues 1:10.

Pig no.	6	7	8
Liver	408	374	389
Kidney	600	400	492

Table 3. 50 per cent elimination time of enzymes calculated from Fig. 1.

Pig no.	79/1	79/7	79/8
GOT	4.0	6.0	5.5
OCT	19.0	17.0	20.0
SDH	1.6	1.8	1.3

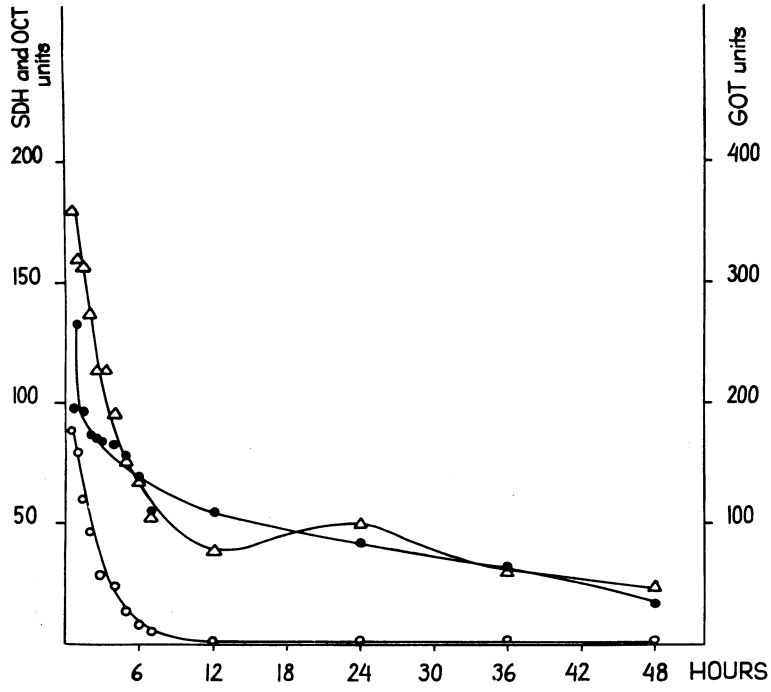

Fig. 1 a. Pig 78/1.

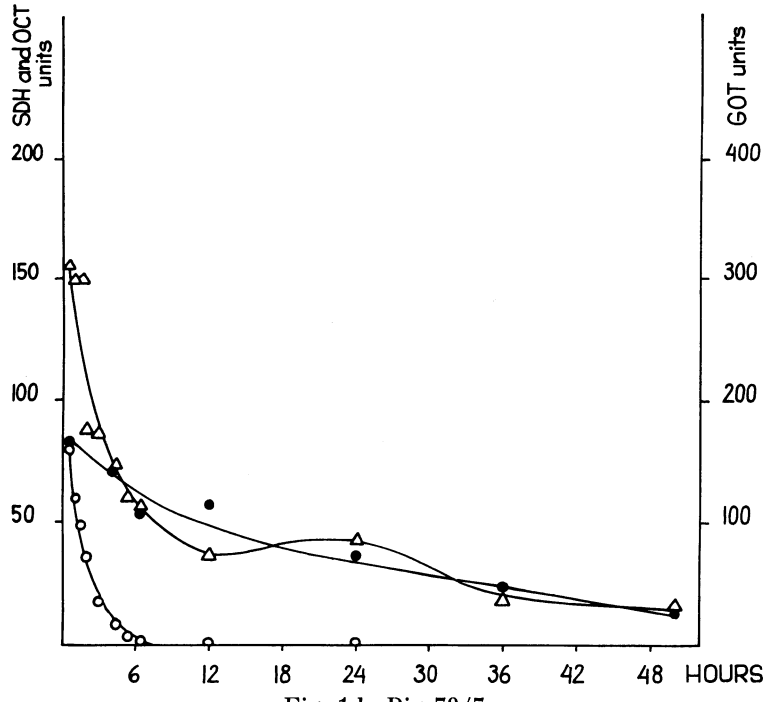

Fig. 1 b. Pig 79/7.

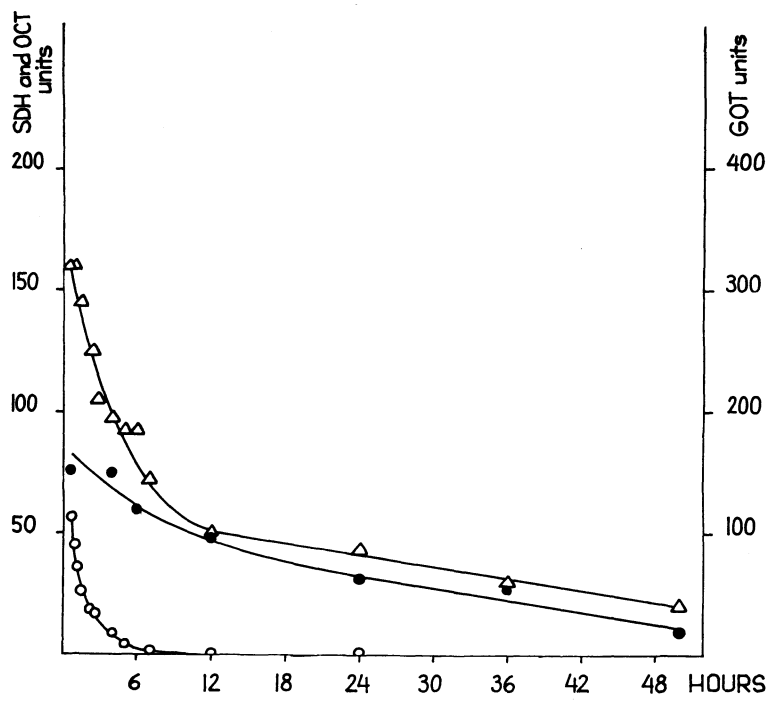

Fig. 1 c. Pig 79/8.

△ GOT, ● OCT, ○ SDH.

F i g. 1. Elimination from venous blood serum of enzymes after intravenous infusion (at zero time) of liver suspension, 1 ml per kg bodyweight, into 3 pigs, weighing about 48.0 kg (pig no. 78/1), 39.5 kg (no. 79/7), and 40 kg (no. 79/8). The SDH activity of suspension was 4800 units.

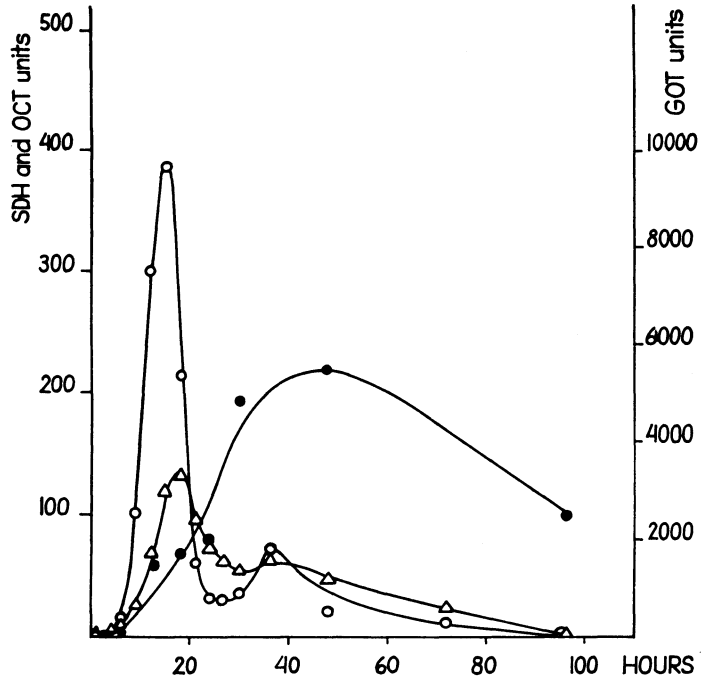

Fig. 2 a. Pig 80/10.

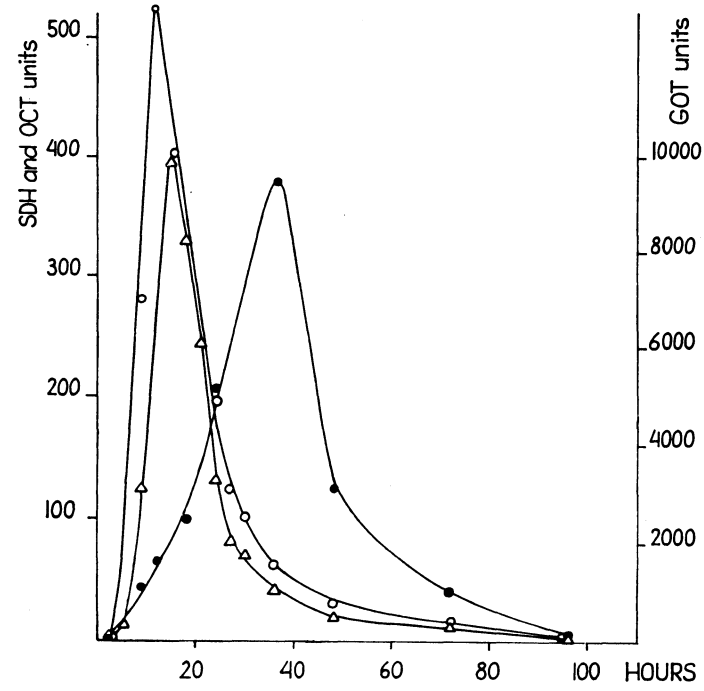

Fig. 2 b. Pig 80/3.

△ GOT. ● OCT. ○ SDH.

Fig. 2. Serum-enzyme activities of 2 pigs which were given carbon tetrachloride at zero time.

The serum-enzyme activity of pigs which had been given carbon tetrachloride is seen in Fig. 2. The pigs were examined for retention of bromsulphalein in serum (5 mg. per kg. body-weight intravenously; elimination time 30 minutes) before the administration of carbon tetrachloride and after 12, 24, and 48 hours, respectively. Two elevations above normal (< 0.3 mg. per 100 ml.) were observed, namely, 1.0 mg. in pig no. 80/10 at 48 hours and 0.7 mg. in pig no. 80/3 at 12 hours. Non-protein nitrogen was determined in serum on the same occasions. No elevations were observed, all values being below 46 mg. per 100 ml. of blood.

The decrease of serum-SDH activity during storage in the refrigerator is shown in Fig. 3. After storage at — 25 °C and at + 20 °C for 24 hours the activities were 88 per cent and 1.4 per cent, respectively, of the original value.

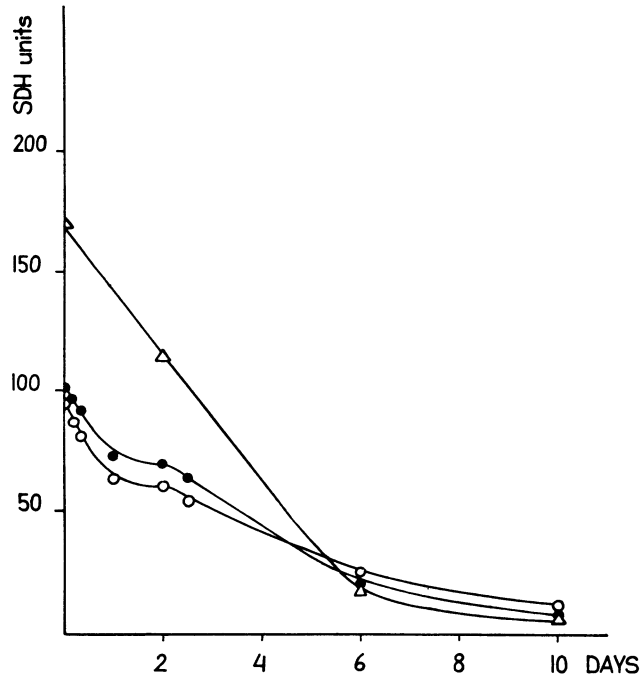

Fig. 3. Decrease of SDH activity at refrigerator temperature (+ 4° C) in three different sera.

DISCUSSION

The question of specificity of a serum-enzyme elevation is of importance in the clinical diagnosis of diseases associated with tissue destruction. In swine medicine reliable liver-tests are very useful (10).

Our results show that in pigs SDH occurs in considerable quantities in the liver and kidney only (Table 1). The activity of the liver is roughly twice that of the kidney. The values are only semi-quantitative, however, since SDH is partly destroyed during the preparation and determination procedure (1).

SDH and OCT are both richly represented in the liver, but the kidneys contain only traces of OCT (13). Therefore, at liver-cell destruction a rise of SDH and OCT, but at kidney-cell destruction a rise of SDH only, should be expected.

The rate of elimination after intravenous infusion of high-enzyme suspensions is also of interest from a clinical point of view. Thus, by examining two or more serum-enzymes whose disappearance rates are known, information about the effect of a therapy can be obtained.

It is apparent that SDH disappears from the circulating serum about twice as fast as GOT and 10 times as fast as OCT (Fig. 1, Table 3). That this is the case also when the enzymes are elevated as a result of destructionof the host's own cells is supported by the curves in Fig. 2. The carbon-tetrachloride-poisoned pigs survived after having shown slight apathy and anorrhexia for about 24 hours, beginning 7—8 hours after administration of the poison. This indicates a fairly slight injury of short duration.

It has been suggested (3, 5) that the primary injury from carbon tetrachloride is an impairment of oxydative phosphorylation and loss of mitochondrial integrity. The first phase of a disturbed distribution of the enzymes studied here may then be that they escape from the damaged mitochondria into the cytoplasm. The second phase would be a diffusion through cell walls into extracellular compartments, including blood. The difference in time between the peak concentrations of SDH, GOT and OCT (Fig. 2) would then be explained by different times required for this diffusion.

Reichard (11) studied GOT and OCT in serum of carbon tetrachloride poisoned dogs. The peak concentration occurred about 2 days after the administration of the poison. GOT was normal after 6—8 days. OCT was still above normal after 14 days. In our

cases the GOT (and SDH) peaks occurred after 15—20 hours and the OCT peak after 36—48 hours. This earlier appearance of maximum concentrations as well as an earlier return to normal levels probably depends on the fact that we used lower doses of carbon tetrachloride (0.25 ml. as against 1 ml. per kg. bodyweight). Species differences may also have played a part.

The liver and kidneys contain about equal amounts of F-1-P-aldolase (Table 2). The liver shows lower activity of aldolase than of SDH. This indicates that SDH is of greater value as a diagnostic enzyme in hepatic disorders. Further determinations of F-1-P-aldolase were therefore not made.

The activity curve of SDH during storage of serum at refrigerator and room temperature shows a rapid decline (Fig. 3). This necessitates immediate determination after the blood specimen has been taken.

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SUMMARY

Sorbite-dehydrogenase (SDH) activity was determined in normal swine serum and tissues. The normal serum-level is 0.44 units (s. d. 0.26). The liver and the kidneys are the only organs that contain considerable quantities. SDH is eliminated from the circulation more rapidly than glutamic-oxaloacetic transaminase (GOT) and ornithine carbamyl transferase (OCT). Carbon tetrachloride poisoning of pigs resulted in a rapid increase of serum-SDH activity.

ZUSAMMENFASSUNG

Studier über die Aktivität der Sorbitdehydrogenase in Blutseren und Geweben normaler Schweinen und nach Tetrachlorkohlenstoff-Vergiftung.

Die Aktivität der Sorbitdehydrogenase (SDH) wurde in Blutserum und Geweben normaler Schweine bestimmt. Der normale Serumgehalt beträgt 0.44 Einheiten (S. D. 0.26 Einheiten). Die Leber und die Nieren sind die einzigen Organen die nennenswerte Mengen enthalten. SDH wird aus Cirkulation schneller ausgeschieden als Glutamin-Oxalessigsäure Transaminase (GOT) oder Ornithin-Carbamyl Transferase (OCT). Tetrachlorkohlenstoff-Vergiftung bei Schweinen gab eine rasche Erhöhung des Serum-SDH-Gehaltes.

SAMMANFATTNING

Undersökningar över sorbitdehydrogenasaktiviteten i blodserum och vävnader hos normala svin samt efter förgiftning med koltetraklorid.

Sorbit-dehydrogenas-aktiviteten (SDH) bestämdes i blodserum och vävnader av normala svin. Den normala serumnivån är 0.44 enheter (s. d. 0.26 enheter). Lever och njurarna är de enda organ som innehåller avsevärda kvantiteter. SDH elimineras ur cirkulationen snabbare än glutamin-oxalättiksyre-transaminas (GOT) och ornitin-karbamyl-transferas (OCT). Koltetrakloridförgiftning hos svin gav snabbt stegrad serum-SDH.

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