(3) The change of the intracellular storage organelles of the platelets is similar to that of the total endogenous 5HT. Thus, after incubation of 6 and 12 h respectively, reserpine diminishes the number of the organelles to 32 ± 4 and $18 \pm 2\%$, whereas imipramine causes no significant diminution (105 ± 4 and $102 \pm 2\%$ compared with controls) (Figure 2).

Discussion. According to the present results, reserpine ($1.6 \cdot 10^{-6} M$), in contrast to imipramine ($10^{-5} M$, markedly decreases the endogenous 5HT of the platelets although both drugs inhibit the uptake of exogenous (radioactive) 5HT to a similar extent. These findings confirm that reserpine and imipramine diminish the uptake of ^{14}C-5HT in platelets by 2 different mechanisms. Reserpine probably interferes with the storage of 5HT in the intracellular organelles leading to a decrease of the endogenous 5HT as well as of the number of the organelles storing 5HT. This has also been shown in earlier experiments in rabbits and guinea pigs to which the drug was administered in vivo[2,3].

Imipramine does not markedly impair the amine storage in the 5HT organelles according to the present experiments. The drug seems, however, to inhibit the 5HT transport through the platelet membrane. Thus, in guinea pig platelets incubated with ^{14}C-5HT, imipramine diminished the formation of ^{14}C-5HT metabolites without interfering with the mitochondrial monoamine oxidase (MAO)[2]. This indicates that the penetration of the amine into the cell is inhibited. In preliminary experiments a similar action of imipramine has been demonstrated in cat platelets.

Since imipramine markedly inhibits the uptake of ^{14}C-5HT by platelets, it probably also interferes with the re-uptake of spontaneously released 5HT by platelets under physiological conditions (e.g. in plasma or Tyrode). According to the present experiments, however, the drug does not markedly affect the stored endogenous amine in the cells. It may therefore be assumed that under physiological conditions spontaneous release of stored 5HT into the extracellular space and re-uptake of the amine into the storage organelles do not occur to a major extent. Interference with the intracellular storage mechanism, e.g. by reserpine, is therefore more effective in depleting the endogenous platelet 5HT than inhibition of the amine transport through the cell membrane as exerted by imipramine.

Zusammenfassung. In isolierten Blutplättchen von Katzen vermindert Imipramin im Gegensatz zu Reserpin das endogene 5-Hydroxytryptamin (5HT) und die 5HT-Speicherorganellen nicht wesentlich, obwohl es wie Reserpin die Aufnahme von exogenem 5HT herabsetzt. Hemmung des Membrantransportes von 5HT durch Imipramin scheint also das intrazellulär gespeicherte 5HT nur relativ wenig zu beeinflussen.

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Comparative Effects of Carbon Tetrachloride and Colchicine on Xanthine Dehydrogenase

Our laboratory has been concerned with changes in serum xanthine oxidase (XO) following CCl_4 administration[1]. It has also been demonstrated that colchicine is able to produce increased levels of rat serum XO[2] and reduced levels of the enzyme in the liver under identical experimental conditions[3].

Experiments on the simultaneous changes in serum and liver enzyme activity after CCl_4 or colchicine administration were undertaken in order to study the disappearance of the enzyme from liver and the possible correlation of these changes with a disturbance in the lipid metabolism under these experimental conditions.

Materials and methods. Adult male Wistar rats ranging in weight from 100–150 g were used throughout. The animals were injected i.p. with a single dose of either CCl_4 (0.1 ml/100 g body weight) or colchicine (0.1 mg/100 g body weight). The colchicine solution contained 20 mg of the alkaloid/100 ml of 6.8% ethanol solution (v/v) and the results presented in this paper for colchicine treated rats have already been corrected for the values obtained for animals injected with a single dose of a 6.8% alcohol solution.

The animals were starved for 20 h, unless otherwise stated. Blood samples were collected by heart puncture under light ether anaesthesia, at the proper time intervals (usually 20 h). In some cases, the liver was removed, weighed, and homogenized in a Warring blender in $0.015 M$ pyrophosphate buffer, pH 8.6, so that the fina homogenate contained 200 mg of tissue/ml of homogenate. Dry weight was determined in all cases.

The xanthine dehydrogenase (XD) activity was measured in 0.5 or 1 ml of clear blood serum incubated with 0.1 ml of a $0.05 M$ hypoxanthine solution as substrate and 0.3 ml of a 0.1% triphenyltetrazolium chloride solution as hydrogen acceptor in an evacuated Thunberg tube.

XO activity was determined manometrically in a Warburg respirometer at 37 °C as described previously by Villela and Mitidieri[4]. XO as well XD activity determinations were carried out in pyrophosphate buffer, pH 8.6.

Iodine value was determined according to Yasuda[5] and total lipids were estimated colorimetrically following the method of Bragdon[6].

Results and discussion. Serum XO and XD activities of normal control rats, of rats treated with CCl_4, and of rats

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treated with colchicine (20 h after the administration of the drugs) are shown in the Table. Average values for total lipids and the iodine value in the serum of control and CCl_4 treated rats are also presented in the Table.

Statistical analysis of the results showed a significant increase in the enzyme activities of rats injected with CCl_4 ($P<0.001$). The iodine value was also increased, reflecting an elevation of the unsaturated fatty acids in these experimental conditions ($P<0.001$). However, total lipids were only slightly increased ($P<0.02$), or unchanged.

To determine the effect of time on the enzyme activity after administration of the drugs, the animals were injected with either CCl_4 or colchicine and the blood was collected from one up to 90 h after the administration of either drug. In both cases, maximum activity in the serum was found 20–40 h after the administration of the drug. The enzyme activity was gradually decreased towards control levels.

In the animals intoxicated with CCl_4 or with colchicine the changes are identical, whether the activities are measured by oxygen consumption (XO) or by the formazan technique (XD) as exemplified in the Figure.

The Figure (upper) shows a decrease in the enzyme activity of the liver which parallels the increase observed in the serum of the animals treated with colchicine. Maximum inhibition was also obtained 20–40 h after the administration of the drug, the time at which peak activity was noted in the serum. In the animals treated with CCl_4, liver XO activity was also increased (Figure, lower), maximum increase being observed 20–40 h after the administration of the drug. Therefore, the effect of CCl_4 upon the liver enzyme was opposite to that observed for colchicine.

The experimental data presented here indicate that increased levels of enzyme activity are observed in the serum of animals receiving CCl_4 or colchicine. Also, it is possible that these increased levels of serum enzyme are somewhat related with the lipid metabolism in these experimental conditions.

The entire mechanism by which CCl_4 produces a fatty liver is still unknown. However, a great number of findings[7,8] suggest that this is due to an impairment in the mechanism by which the liver secretes large amounts of triglycerides into the blood. SPITZER and MILLER[9] showed that there are increased levels of plasma non-esterified fatty acids (NEFA) following CCl_4 poisoning. This elevation in plasma is also observed following the administration of colchicine[10]. MITIDIERI and AFFONSO[11] presented some evidence that there is a correlation between the XD activity and the iodine value of blood serum lipids in these experimental conditions. Another piece of evidence of the relation of XD activity with the lipid metabolism was obtained earlier[12], showing that XD activity was confined to the lipoprotein fractions of serum separated by paper electrophoresis.

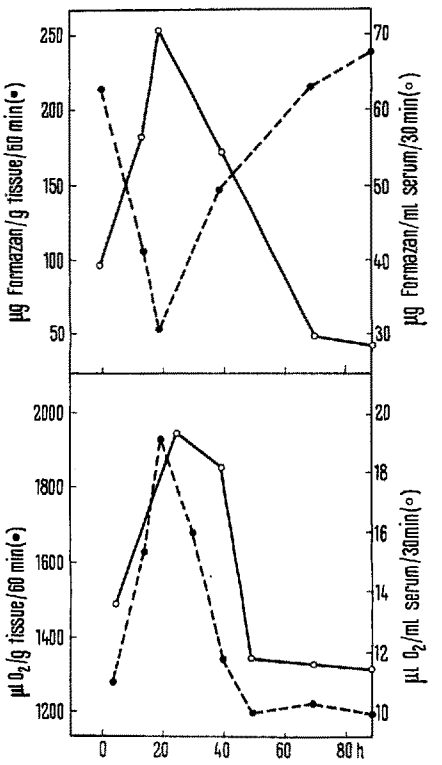
Although all the data presented suggest that XD is somewhat correlated with the lipid metabolism, it appears unlikely that the blockage in the secretion mechanism as proposed by RECKNAGEL et al.[7] was responsible for the increased XD levels observed in our experimental conditions. One of the explanations for these elevated levels could be a de novo synthesis of the serum enzyme as an alternative to overcome the liver cell injury.

Another possible explanation is a release of enzyme by cellular disruption. This is supported by the fact that

Variations in xanthine oxidase and dehydrogenase activities, iodine value and total lipids in serum

Determinations	Control animals	Treated with CCl_4 0.1 ml/100 g body weight	Treated with colchicine 0.1 mg/100 g body weight
XO activity[a]	13.1 ± 1.8 (16)	19.5 ± 1.7 (13)	20.9 ± 5.4 (8)
XD activity[b]	17.2 ± 3.0 (14)	27.1 ± 2.3 (7)	28.1 ± 3.0 (8)
Iodine value	84.2 ± 11.2 (10)	138.5 ± 16.9 (12)	
Total lipids[c]	339.9 ± 39.4 (10)	380.0 ± 29.6 (10)	

The numbers in parenthesis indicate the number of sets of 3 rats used in each group. All values represent mean ± standard deviation. S.D. = $[(x-\bar{x})^2/(n-1)]^{-1/2}$. [a] μl O_2/ml serum/60 min. [b] μg formazan/0.5 ml serum/30 min. [c] mg/100 ml serum.



Simultaneous changes in serum (o—o) and liver (•—•) XD and XO activities after the administration of colchicine (upper) and after the administration of CCl_4 (lower). Each point represents the mean value obtained after duplicate determination in 3 sets of 3 rats each.

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XD is a non-particulate cytoplasmic enzyme[13] and could very well be capable of passing through an abnormal cell wall. This mechanism could explain the rise observed in serum XD during colchicine treatment of rats once a decrease was observed in the enzyme content of the liver. Nevertheless, this could not entirely explain the increase in serum enzyme activity following CCl_4 since the liver enzyme is also activated, both in vivo and in vitro, by CCl_4[11]. This is possibly due to a direct effect of CCl_4 on the lipid moiety that may be associated with the enzyme molecule[14].

Résumé. Une augmentation de l'activité de la xanthine déshydrogénase et de la xanthine oxydase du sérum des animaux ayant reçu du tetrachlorure de carbone ou de la colchicine a été observée. Il nous semble que cette augmentation soit en relation avec des variations du métabolisme lipidique, puisque dans les conditions expérimentales mentionnées, des changements du taux des acides gras non saturés ont également été constatés. Les auteurs discutent certaines hypothèses sur les variations simultanées de l'activité des enzymes du foie et du sérum dans des animaux injectés.

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Biochemical Laboratory, Instituto Oswaldo Cruz, Rio de Janeiro (Brazil), 14th September 1966.

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[14] This research was supported by grants from the Conselho Nacional de Pesquisas (Brazil).

Dati preliminari sulla presenza di zinco nel veleno cutaneo di anfibi

Recenti dati sperimentali di COLBURN e MAAS[1] hanno dimostrato come il rapporto tra adrenalina e noradrenalina e ATP possa stabilirsi mediante un metallo chelante con formazione di complessi ternari.

Impegnati in una ricerca sulla presenza di ATP nel veleno cutaneo degli anfibi[2] ci siamo prospettati la possibilità che anche tra l'ATP e le varie sostanze 5-idrossindoliche presenti nel veleno stesso potessero almeno in parte attuarsi condizioni simili. Pertanto abbiamo voluto cominciare a raccogliere qualche dato sulla presenza eventuale di metalli cui potesse essere attribuita una funzione del genere; la nostra attenzione si è in primo luogo rivolta alla dimostrazione dello zinco anche perchè per questo metallo esistono delle buone possibilità di rivelazione istochimica[3].

Intendiamo qui render conto di qualche risultato preliminare raccolto in attesa di poter dare più ampio sviluppo alle nostre osservazioni.

Già alcune prove preliminari condotte su materiale fresco di veleno di *Salamandra maculosa, Bombinator pachypus* e *Triton cristatus* sottoposto alla reazione al ditizone secondo il procedimento di FISCHER[4] avevano dimostrato positività intensa per lo zinco.

Successivamente, seguendo il procedimento di MALMSTRÖM[5] abbiamo seguito spettrofotometricamente la reazione al ditizone sia su standard di $ZnCl_2$ e $CuCl_2$ e così pure su ceneri ottenute da veleno di *S. maculosa* e *B. pachypus*.

I risultati sono riportati nel grafico di Figura 1. Si osserva un picco attorno a 530 nm per la reazione Ditizone-Zn; cui corrispondono analoghi picchi per i veleni di *Salamandra* e *Bombinator*. Per *Bombinator* è evidente altresì un picco attorno a 420 nm che poteva far pensare alla presenza di Cu, come si vede bene nel grafico per la reazione Ditizone-Cu; circa la presenza di questo metallo intendiamo occuparci in seguito in altra sede.

Per confermare i dati relativi alla presenza di Zn ottenuti spettrofotometricamente e volendo estendere anche ad altre specie le osservazioni, abbiamo sottoposto campioni incineriti a + 600 °C all'esame polarografico. Le curve polarografiche sono state registrate in soluzione tiocianica tamponata a pH 6,8 complessando il ferro eventualmente presente con NaF[6].

Per ora abbiamo avuto dati nettamente positivi con metodo polarografico nelle seguenti specie: *T. cristatus*, *S. maculosa* e *B. pachypus* come dimostrano i grafici in Figura 2.

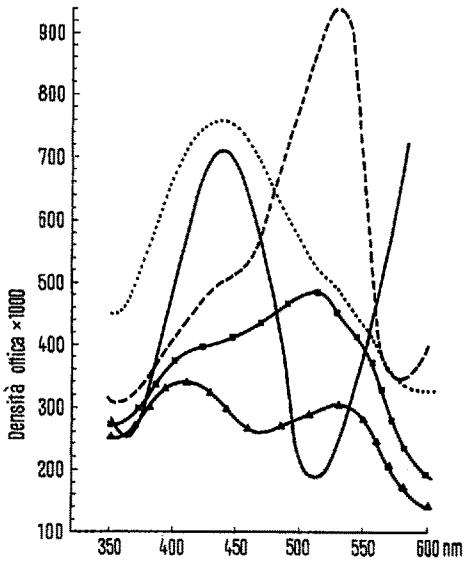

Fig. 1. Determinazione spettrofotometrica dello Zn mediante reazione al ditizone. ———, Ditizone; ·····, Ditizone + $CuCl_2$; – – – – – –, Ditizone + $ZnCl_2$; ■—■—■, Ditizone + veleno *Salamandra maculosa*; ▲–▲–▲, Ditizone + veleno *Bombinator pachypus*.

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[6] Ringraziamo vivamente il Prof. V. RIGANTI dell'Istituto di Chimica Generale della nostra Università che gentilmente ha eseguito le determinazioni polarografiche.