products present. After 8 weeks' storage, TLC was rerun, spotting 50 mcg. (as benzoyl peroxide based upon 5% labeled concentration) of each sample, together with known concentrations of benzoic acid. Estimation of the spots under long-wave UV light (366 mμ) indicated approximately 10 to 13 mcg. of benzoic acid present (20 to 26% of the labeled benzoyl peroxide concentration). Table III(B) illustrates the findings.

These results confirm the spectrophotometric findings of Gruber and Klein (1) who reported considerable loss of benzoyl peroxide dispersed in pharmaceutical preparations but not in benzoyl peroxide-calcium phosphate powder.

While benzoic acid was the only significant deteriorative product separated from benzoyl peroxide pharmaceuticals, the IR spectra and earlier polarographic studies (1) prove the presence of intermediate degradation products. While benzoyl peroxide-calcium phosphate powder exhibited no breakdown after 2 years at room temperature, once this powder was dispersed in its vehicle, decomposition followed closely with other commercial preparations, remaining stable within the period specified in its labeling.

SUMMARY

Previous spectrophotometric and polarographic analyses indicating substantial loss of benzoyl peroxide in pharmaceuticals have been corroborated. The inadequacy of conventional peroxide titration procedures is confirmed.

IR spectra of benzoyl peroxide lotions prove the formation of benzoic and/or related acids as well as aldehydes. TLC studies with semiquantitative analysis indicates degradation of benzoyl peroxide preparations stored at room temperature, with benzoic acid and/or related acids separated. Dry powdered compositions of benzoyl peroxide (benzoyl peroxide-calcium phosphate), however, indicate no breakdown after extended storage.

This study demonstrates that pharmaceutical preparations containing benzoyl peroxide possess limited shelf-life and should bear expiration dates.

While determination of certain specific degradation products in benzoyl peroxide pharmaceutical preparations has herein been determined, further investigations are being conducted in an attempt to ascertain the chemical configuration of the intermediate unstable and metastable decomposition products.

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Serum Enzyme Patterns in Acute Poisoning with Organochlorine Insecticides

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Abstract □ The effect of the administration of single, oral, convulsive doses of DDT, aldrin, dieldrin, or endrin on the activity of selected serum enzymes was documented in the intact albino mouse. Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and lactic dehydrogenase levels in the treated animals were significantly increased above those seen in animals receiving no treatment or the vehicle only. The observed serum enzyme pattern apparently indicates that the insecticides studied induce a degree of hepatotoxicity. This may be seen after a single exposure, when the animal has reached the convulsive stage.

Keyphrases □ Organochlorine insecticides—acute toxicity □ Enzyme systems, serum—organochlorine insecticide effect □ Electrophoresis—analysis □ UV spectrophotometry—analysis

A wide array of enzymes has been demonstrated in the blood of mammals. A number of these have their loci of action in this tissue. The greatest number of these biocatalysts, however, are elaborated and have their site of action in one or more other tissues. No matter their source, the concentration and distribution of enzymes in blood as well as other tissues or organs reflect the functional, morphologic, and biochemical status of their point of origin and activity. This distribution constitutes a characteristic pattern which may be considered an enzyme profile. These profiles may be altered by changes in metabolism, cellular integrity, membrane permeability, exogenous chemicals, stress, or a combination of these. Often the alteration in enzyme pattern or the concentration and distribution of individual enzymes is seen well before morphologic change is evident microscopically.

For some years, enzyme concentration in plasma or serum has been used in the diagnosis of disease states. Relatively more recently, changes in enzyme concentration have been employed in the evaluation of toxicodynamic response. Within the past few years, toxicologists have become interested in the response of individual enzymes or groups of enzymes to toxic insult. Although the occurrence of a change in concentration of a specific enzyme might reflect specific or general tissue damage or functional derangement, changes in the profiles exhibited by groups of enzymes might well be characteristic of specific toxicants or classes of toxicants. Additionally, such changes might be of value in elucidating modes of action of poisons. With

this in mind, it was decided to look at the activity of some of the more ubiquitous enzymes in the sera of mice acutely poisoned with selected organochlorine insecticides.

Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum lactic dehydrogenase (SLDH) were chosen for initial investigation because of data already available regarding these enzymes in relation to carbon tetrachloride (CCl_4) intoxication. In contrast to CCl_4 and other chlorinated hydrocarbon solvents, the organochlorine insecticides are CNS stimulants rather than depressants. Like CCl_4, however, these insecticides do exhibit varying degrees of hepatotoxicity. It was of interest, therefore, to see whether this group of compounds would induce changes in the enzyme profile similar to that seen in CCl_4 poisoning.

MATERIALS AND METHODS

Test Animals—The test animals used in this study were 9-week-old male albino mice of the Swiss-Webster strain. After arrival, a 3-day period was allowed for acclimatization. During this time, they were weighed and examined (daily) for health status, culled where indicated, and divided randomly into six test groups of 30 animals each. The weighing and culling were repeated on succeeding days. Animals exhibiting a 10% or greater loss of weight were replaced from a reserve group. Animals were maintained on Purina mouse breeder chow and water *ad libitum*. Sixteen hours prior to dosing, all animals were taken off feed. However, water was not withdrawn.

Test Compounds—This report is part of an ongoing study of a selected series of enzymes. Corn oil was used as the solvent for the oral administration of the toxicant in all phases of the study. The use of this particular oil in this experiment is simply a continuation of the standard practice in this investigation.

The test toxicants used in this study were technical grade DDT, aldrin, dieldrin, and endrin, containing 98–99% of active ingredient, as supplied by the manufacturer.

Dosing—Insecticides were dissolved in corn oil and administered *per os* using a stainless steel oral feeding tube and a precision grade tuberculin syringe. Doses were so calculated that the total volume administered was kept uniform at 0.4 ml. Of this, 0.1–0.15 ml. constituted a wash to flush the insecticide solution into the stomach. DDT was administered as a 4% solution; the other pesticides, as 1% solutions of the 100% active compounds. Test animals were dosed with convulsive doses of DDT, aldrin, dieldrin, and endrin as indicated in Table I.

After dosing, animals were placed in individual cages for observation. As an animal went into convulsions, it was decapitated and exsanguinated. Blood was collected in siliconized tubes and allowed to clot at room temperature. Each time a dosed animal was sacrificed, an untreated animal and an animal dosed with vehicle only were sacrificed and their blood collected in a similar fashion. Sera from three animals, similarly dosed, were pooled and used for each determination of SGOT, SGPT, and SLDH.

Enzyme Assays—Serum lactic dehydrogenase was assayed by measuring the conversion to pyruvate to lactate as indicated by the oxidation of $NADH_2$ to NAD (1). Serum glutamic oxaloacetic transaminase was determined by measuring the oxidation of $NADH_2$ to NAD using malic dehydrogenase (MDH) as the indicator enzyme and oxaloacetate as the substrate (2). Serum glutamic pyruvic transaminase was assayed by measuring the conversion of $NADH_2$ to NAD using pyruvate as the substrate and LDH as the indicator enzyme (3). All assays were carried out at 25°. One-half milliliter of serum was used for the determination of SGPT and SGOT, 0.1 ml. for the assay of SLDH. Reaction products were measured at 340 mμ in a spectrophotometer (Beckman DU) using a silica cell with a 1-cm. light path. Enzyme activity was expressed in milli-units (thousandths of an International Unit). An International Unit is defined as the amount of enzyme converting 1 μmole of substrate per minute at 25°.

The standard error of the mean was calculated for each set of enzyme values. The data was examined for significance using the Student's *t* test. With the exception of the transaminase levels seen in SGOT and SGPT in endrin and dieldrin poisoning, all treatments *versus* each set of controls, differences between treatments, and differences between controls were found to be highly significant. This difference amounted to 0.001 in all instances with three exceptions. Differences were seen in SLDH values as follows: (*a*) no treatment *versus* DDT, 0.05; (*b*) aldrin *versus* dieldrin, 0.005; (*c*) dieldrin *versus* endrin, 0.025. No difference in significance was noted in the transaminases in endrin and dieldrin poisoning.

RESULTS

A consideration of the data presented in Table I reveals that the vehicle had a moderate but significant effect on enzyme activity in the serum. The SGOT activity in the animals dosed with the vehicle only, was some 50% greater than that seen in the untreated animals. SGPT, under the same conditions, showed an increase of only 17%. After the administration of corn oil, SLDH activity in the serum decreased 13% compared to animals receiving no treatment at all.

Treatment with DDT induced increases of 43, 62, and 22% in SGOT, SGPT, and SLDH activity, above that seen in animals dosed with the vehicle only. When compared to the untreated controls, the observed increases amounted to 114, 99, and 5% for the same enzymes.

Dosing with aldrin increased SGOT, SGPT, and SLDH activity by 201, 159, and 41%, respectively, compared to the test animals receiving no treatment. When compared to the vehicle controls, the increases observed in enzyme activity amounted to 100, 121, and 61% for SGOT, SGPT, and SLDH, respectively.

SGOT and SGPT activities were increased 160 and 133% (in that order) with respect to the untreated animals, after treatment with dieldrin. In the case of SLDH, the increase was 27%. Comparison of insecticide-treated animals with those receiving the vehicle only revealed increases amounting to 74, 98, and 48% for SGOT, SGPT, and SLDH, respectively.

Dosing with endrin induced increases of 109, 91, and 21% in SGOT, SGPT, and SLDH activity compared to the untreated controls. When compared to the animals receiving the vehicle only, these figures amounted to 40, 63, and 39% for the same animals.

DISCUSSION

The presence of enzymes of tissue metabolism in normal serum was demonstrated some 25 years ago (4). They apparently have no function in the blood since their substrates are absent from this tissue. The mechanisms whereby tissue enzymes are extruded into the blood have not been elucidated. It would appear that this efflux cannot be attributed to a single mechanism.

Table I—Serum Enzyme Activity in Insecticide Poisoning

Treatment	Dose, mg./kg.	SLDH[a]	Standard Error	SGOT[a]	Standard Error	SGPT[a]	Standard Error	SGOT:SGPT (ratio)
Untreated control	0	143.8	2.28	16.4	0.40	17.5	0.43	0.94
Vehicle control	(0.4 ml.)	124.8	1.62	24.5	0.29	20.5	0.22	1.19
Endrin	20	173.6	1.91	34.3	0.21	33.4	0.50	1.05
DDT	500	151.6	3.20	35.1	0.73	33.4	0.43	1.03
Dieldrin	40	184.0	3.28	42.6	0.34	40.7	0.81	1.05
Aldrin	70	202.2	4.11	49.2	0.84	45.3	0.59	1.09

[a] Milli-units/ml. of serum (mean of 10 assays). 1 milli-unit (m.u.) = 0.001 I.U.

A large body of evidence is available indicating that increases in serum enzyme activity are directly related to cellular damage. Excellent correlation between enzyme level and dose of toxicant administered has been demonstrated (5). Damaged organs have been found to show a decrease in enzyme activity (6). The characteristics and proportions of the enzymes from such organs agree well with those concomitantly found in the serum (7). It has been demonstrated that there is good correlation between the extent of necrosis and the rise in serum enzyme activity (8, 9). However, significant increases in serum enzyme levels have been noted following cell damage which could not be demonstrated morphologically (10). Zierler (11) has reported that membrane permeability plays an important role in the efflux of enzymes into the serum. *In vitro* studies (12) indicate that transaminases may be liberated from apparently vital cells. This is borne out by the observation that SGOT activity is increased after exercise.

It would thus appear that the release of intracellular enzymes follows a disruption or change in cellular metabolism or activity. The change from the normal enzyme profile does not reflect an uncoordinated flow of individual enzymes from the affected cell. Rather, the distorted pattern mirrors metabolic changes induced in a tissue by toxic insult, stress, or a pathologic process. The data presented in this report indicate that a single dose of a toxicant can induce a significant rise in serum enzyme levels.

The serum enzyme activity in the test animals, dosed with vehicle (corn oil) only, was found to be significantly different from that seen in those receiving no treatment at all. Englhardt-Golkel *et al.* (13) observed changes in glycolytic serum enzymes, in man, after the ingestion of a rich meal. These changes could not be attributed to technique or the method of assay. Since increases as well as decreases in the same enzyme were seen in different subjects, they did not investigate the matter further. The authors' findings are of particular interest, since the transaminases were increased in all test animals in this laboratory, while SLDH was concomitantly decreased. These observations were found to be significant at the 0.001 level. It may be that this phenomenon is due to handling or the stress associated with the administration of the corn oil. The possibility that SLDH is decreased in stress is apparently not tenable in this instance, since the administration of vehicle plus insecticide overcame the initial fall in enzyme level and induced a statistically significant increase in activity above that seen in either the vehicle-treated or untreated animal. That an apparently inocuous vehicle may be a factor in physiologic response has some interesting implications in toxicologic evaluation. These observations merit further investigation. A study of these observations is in the final planning stage at this time.

Both of the transaminases studied are widely distributed in mammalian tissue. High levels of SGOT are considered indicative of acute hepatic necrosis. High levels have been observed after the administration of hepatotoxins such as CCl_4 (14). However, high SGPT levels are considered to be a more specific indication of liver damage. It has been reported that SGPT levels are higher than SGOT in chemical intoxication (15). The data reported here indicate essentially similar or lower levels of SGPT after dosing with organochlorine insecticides.

Lactic dehydrogenase and glutamic pyruvic transaminase are found in the cytoplasm only, in contrast to glutamic oxaloacetic transaminase which is present in the mitochondria as well. Mitochondrial GOT would not be expected to leave these organelles under physiologic conditions. GOT concentration does, in fact, appear in the same proportion in both the serum and the cytoplasm. In liver cell damage, where there has been a loss of mitochondrial integrity, one would expect to see extravasation of mitochondrial GOT into the cytoplasm and eventually into the serum. Since the absolute concentration of this enzyme, in the cell, is greater than that of GPT, one might expect to see increased levels of SGOT in hepatocellular damage.

Data from a study by Asada (16), as well as the findings presented in this report, bear out this line of reasoning. Asada studied transaminase activity in experimental liver damage induced by carbon tetrachloride. Twenty-four hours after a single intramuscular dose of CCl_4, he found serum transaminase activity had been increased 118 and 117% in the case of GOT and GPT, respectively. The SGOT:SGPT ratio was found to be 1.37 compared to 1.27 for the untreated animals—representing an 8% increase in the SGOT:SGPT ratio.

In the study reported here, it was found that during the onset of convulsions, the SGOT:SGPT ratio was increased above the normal value of 0.94 by some 10 to 15% depending upon the insecticide used. Serum transaminase activity increased 109 to 200% in the case of GOT and 91 to 159% in the case of GPT, depending upon the insecticide used.

Asada's data is based on observations made 24 hr. after a single intramuscular injection of CCl_4. Data in this report are based on the documentation of serum enzyme levels, at the onset of convulsions, after the administration of a single oral dose of either DDT, aldrin, dieldrin, or endrin. The findings, however, appear to be comparable. SGOT was increased to a greater extent than SGPT.

In contrast to Wroblewski's report on human clinical material (17), Asada (18) has presented evidence that SGPT is lower than SGOT activity in acute liver damage. In discussing transaminase levels in nonviral hepatitis, Steigmann *et al.* (19) commented that while other workers had reported that SGPT activity was greater than that of SGOT, in such instances, their own findings were to the contrary.

The percent elevation of SLDH was consistently lower than that of SGOT or SGPT. This is in keeping with the well-documented observation that the rise in SLDH in toxic hepatitis is neither as steep nor as great as that of the transaminases.

It has been reported that increased metabolic activity may be a factor in the elevation of enzyme activity in the serum. The authors found that the activity of SLDH, SGOT, and SGPT was significantly greater after aldrin administration than after dosing with any of the other insecticides. Since aldrin undergoes epoxidation as well as detoxication, one would expect greater metabolic activity in the organism's response to this toxicant.

Sova (20) studied the effect of selected organochlorine insecticides on lactic dehydrogenase, *in vitro*. He found that DDT, aldrin, dieldrin, and endrin inhibited rabbit muscle lactic dehydrogenase, when determined by direct measurement of DPN reduction. The authors' data on total SLDH, *in vivo*, indicate a consistent increase in SLDH activity, ranging from 29.5% in the case of DDT to 62% in the case of aldrin. The difference in findings may be due to the test systems employed.

In addition to a consideration of the difference in the test system, other reasons for the observed distinction may be proposed. In the intact animal, the symptomatology seen in poisoning by organochlorine insecticides though referable to CNS stimulation is characterized by hyperactivity and convulsions. It has been shown that increased muscular activity will provoke enzyme leakage into the serum. Thus one would expect elevated SLDH levels under such circumstances. The authors' data support this. It is possible that a decrease in one of the LDH isoenzymes might, under physiologic conditions, be compensated by increased stimulation followed by an overshoot of one or more of the others.

Luckens (21) reported changes in the electrophoretic patterns in sera from mice poisoned with organochlorine insecticides. A splitting or smearing of the α-2 globulin band was seen. This was reflected, generally, by an increase in the concentration of this fraction in the male and a decrease in the female test animal. No significant change was seen in the α-1 globulins in either sex, compared to the controls. The β-globulin fraction showed a decrease, compared to the controls in both sexes.

These observations are of interest in connection with the data presented here. Glutamic oxaloacetic transaminase migrates, electrophoretically, as a slow α-2 globulin; glutamic pyruvic transaminase has a mobility comparable to β-globulin (22). Lactic dehydrogenase, as might be expected, migrates with several protein fractions. It would appear that the distortion in the transaminase-LDH pattern is reflected in the protein pattern seen on electrophoresis.

Data from this laboratory, though limited, indicates that the organochlorine insecticides studied have an apparent degree of hepatotoxicity as reflected by their serum enzyme patterns. This may be seen after a single oral dose when the test animal has reached the convulsive stage. The SGOT:SGPT:SLDH profile is similar to that seen in CCl_4 a chlorinated hydrocarbon hepatotoxin which is a CNS depressant rather than a stimulant, as in the case of the insecticides studied. This would seem to indicate that the hepatic and CNS effects are probably not related.

SUMMARY

Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and lactic dehydrogenase were assayed in the sera of mice receiving

single, oral, convulsive doses of DDT, aldrin, dieldrin, or endrin. SGOT, SGPT, and SLDH levels in the treated animals were significantly increased above those seen in either the undosed or vehicle-treated controls.

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Simultaneous Extraction of Tissue Norepinephrine and Serotonin

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Abstract □ A new procedure for the extraction of tissue monoamines combines the homogenization and butanol extraction steps. In addition to the significant reduction in the time necessary for extraction which is characteristic of single-extraction procedures, greater extraction efficiencies are obtained. Hence, the absolute amount of amine available for spectrophotofluorometric determination is greater and the potential error arising from correction for losses during extraction is reduced. The procedure has been routinely employed in this laboratory for more than 2 years, and has proven to be reliable.

Keyphrases □ Serotonin, norepinephrine—simultaneous tissue extraction □ Tissue extraction—serotonin, norepinephrine, radioactive □ Scintillometry, liquid—analysis

Since the report of Shore and Olin (1) virtually all methods of tissue norepinephrine determination have incorporated extraction of the amine from a tissue homogenate prepared in dilute hydrochloric acid. This type of extraction was also used by Wiegand and Perry (2) to determine tissue epinephrine, serotonin, DOPA, and dopamine in addition to norepinephrine.

Shore and Olin (1) recognized that the efficiency of norepinephrine extraction into butanol from a hydrochloric acid homogenate was low. In addition, the homogenate–butanol extraction phase of the procedure was time consuming. Callingham and Cass (3) also observed that destruction of amine due to local overheating during homogenization in glass, and pipeting errors due to homogenate frothing were disadvantages inherent in this procedure. They avoided these problems by pulverizing the frozen tissue in a punch press. The pulverized tissue was then added to a salt-saturated butanol–0.01 *N* hydrochloric acid system for extraction. Hence, while losses of amine prior to extraction were minimized, total recovery of amine remained poor due to the unfavorable partition coefficient of catecholamines in the hydrochloric acid–butanol system. Chang (4) developed a procedure in which homogenization was performed in acidified butanol. This procedure had the advantage of simplicity by combining the homogenization and extraction steps, but the overall recovery of catecholamines, although improved over previous methods, was still poor. Fleming *et al.* (5) developed a procedure in which homogenization was performed in acetone, and the amines were then transferred into acidified butanol. While extraction efficiency was apparently improved and losses of amines were minimal, the procedure is somewhat complex and relatively time-consuming.

The present procedure was developed in an attempt to prevent the loss of monoamines due to factors observed by Callingham and Cass (3), to improve the