1.8N H_2SO_4[15]; any protein which precipitated was removed by centrifugation. The tubes were then left at room temperature for 10 min to allow the yellow colour to develop. The colour which developed was read at 390 nm. Protein determinations were made by the method of LOWRY, ROSEBROUGH, FARR and RANDALL[16], using bovine serum albumen Fraction V as a standard.

All solutions were made up in glass distilled water. All inorganic salts were present as chlorides and were AnalaR grade. Histidine, ATP, BSA and ouabain were obtained from Sigma Chemical Co. It is a pleasure to acknowledge the gifts of Rontyl (E. R. Squibb and Sons), ethacrynic acid (Merck, Sharpe and Dohme) and Lubrol (I.C.I. Dyestuffs Division).

The results are shown in the Table. Considering first the preparation obtained by the extraction method developed for nervous tissue (Expt I). These results show that the preparation was stimulated by Mg^{2+} alone, being unaffected by the further addition of K^+ and slightly inhibited by the addition of Na^+ (about 25%). This basic Mg^{2+} ATPase activity is also reduced (about 10%) when both sodium and potassium ions are present. It would seem that no synergystically stimulated Na^+-K^+-Mg^{2+} ATPase is present in this preparation; however that addition of ouabain further inhibits the enzymic splitting of ATP when both Na^+ and K^+ are present, although it did not affect enzyme activity when only one of the monovalent cations is present. Thus it can therefore be inferred that a ouabain sensitive component is present (i.e. a Na^+-K^+-Mg^{2+} ATPase). It has a fairly low activity of 5.3 nmoles Pi/mg protein/min in comparison with the Mg^{2+} ATPase which was 29.3 nmoles Pi/mg protein/min. Similar results have been obtained in our laboratory from studies on insect muscle (Wareham, A.C., unpublished) and mammalian muscle (Radcliffe, M.A., and Parkin, A.C., unpublished) and can be explained by sodium ion inhibition of the basic Mg^{2+} ATPase activity. Such inhibition would mask the coupled stimulation when both Na^+ and K^+ are present if the Na^+-K^+-Mg^{2+} ATPase proportion of the total ATPase activity is relatively small.

In the second series of experiments (II-V) in which the sodium iodide extraction method was used a very different picture emerges. This technique has been shown to proportionally increase the activity of the Na^+-K^+-Mg^{2+} ATPase as compared with the Mg^{2+} ATPase in other preparations[14], and has produced the same effect in our work. This is most obvious in the *Schistocerca* preparations, where the basic Mg^{2+} ATPase is about 5–10 nmoles Pi/mg protein/min and the activity stimulated by the addition of Na^+ and K^+ is about 65 nmoles Pi/mg protein/min. The basic Mg^{2+} ATPase activity is not stimulated by the addition of either monovalent cation alone. Similar results have also been obtained with *Jamaicana* although the stimulation due to Na^+ and K^+ is only about 100%. In both cases, however, this stimulated activity was completely abolished in the presence of $10^{-3}M$ ouabain. This preparation would therefore seem to fulfill the requirements of a classical Na^+-K^+-Mg^{2+} ATPase, that is it is synergystically stimulated by sodium and potassium and inhibited by ouabain. It is notable that ethacrynic acid, which is not such a potent inhibitor as ouabain[17–19], inhibits the stimulated activity by only about 30%, that being about the same as described for kidney cortex[19]. The addition of Rontyl, a carbonic anhydrase inhibitor, is even less potent, and in 1 of the 2 experiments where it was used it caused no inhibition.

It is tempting to speculate that the differences in the proportion of the Na^+-K^+-Mg^{2+} ATPase activity to the basic Mg^{2+} ATPase in the preparations from *Schistocerca* and *Jamaicana* may well reflect the known differences in the ability of the 2 species to retain water. The locust is suited to live in dry conditions and has a highly efficient means of water retention. By contrast *Jamaicana* requires a regular supply of water in the diet and presumably is less well able to resorb water.

Zusammenfassung. Die Präsenz einer charakteristischen Na^+-K^+-aktivierbaren ATPase (E.C. 3.6.1.3) wurde erstmals in einer mikrosomalen Fraktion des Enddarmes und der malpighischen Gefässe bei der Wüstenschrecke *Schistocerca gregaria* und der Laubheuschrecke *Jamaicana flava* nachgewiesen.

A. J. PEACOCK, K. BOWLER and J. H. ANSTEE

Department of Zoology, University of Durham, South Road, Durham City (England), 3 January 1972.

[15] A. ATKINSON, Ph. D. Thesis, University of Manchester (1970).
[16] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[17] P. W. DAVIS, Biochem. Pharmac. *19*, 1983 (1970).
[18] A. ASKARI and D. KOYAL, Biochim. biophys. Acta *225*, 20 (1971).
[19] J. S. CHARNOCK, H. A. POTTER and D. MCKEE, Biochem. Pharmac. *19*, 1637 (1970).

Enzymic and Behavioural Changes Induced in Mice Fed Polychlorinated Biocides Followed by Starvation

Polychlorinated biocides are mainly stored in fat tissue[1], but a certain amount is metabolized by the microsomal cytochrome P-450 complex[2] which is sensitive to dietary factors, i.e. ethanol[3,4] and starvation[4]. These factors exert either a protracted or a more acute influence. Since most studies on the activity of the P-450 complex due to deficiency states have concerned permanent or substantial lack of one or more dietary factors[4,5], the present communication intends to relate previous intake of polychlorinated biocides to a syndrome following acute starvation of the animals.

Material and methods. Two-month-old female mice (33g) were divided into groups and fed ad libitum for 7 days on a diet optimal in all respects: group 1: without any additions, group 2: was fed the diet of group 1 containing 1500 ppm DDT and group 3: fed the diet of group 1 but containing 1500 ppm γ-hexachlorocyclohexane. After 7 days feeding half of the animals of group 1 and 2 were starved for 4 days. The hexachlorocyclohexane-fed mice were killed without starvation after 7 days feeding due to high mortality (40%).

Chemicals: Glucose-6-phosphate and NADP were from Boehringer, Germany. DDT (dichloro-diphenyltrichloroethane) was from Struers Co., Copenhagen, γ-hexachlorocyclohexane (Lindan) was from Ferrosan Ltd., Copenhagen. Before use, these 3 substances were recrystallized twice from ethanol. All other chemicals were of highest obtainable purity from British Drug Houses, Poole, U.K.

Methods: Animals were killed by a blow on the neck and cytoplasmic fraction of livers was obtained as previously described[3]. The activity of the cytochrome P-450

Liver weights, total protein of the cytoplasmic fraction of liver and the *p*-nitroanisole *O*-demethylation activity of mouse liver microsomal fraction from animals intoxicated with DDT or Lindan

Pretreatment	Liver weight (g)	Total protein of cytoplasmic fraction (mg)	P-450 activity Specific activity (nmoles/min/mg protein)	No. of mice	Death rates (days) 7	11[a]	12[b]
Control	1.73±0.16	30.4±4.3	9.79±0.66 (8)	8	0/8	—	0/8
Lindan	1.76±0.05	28.8±1.1	23.58±0.35 (9)	15	6/15	—	—
Control starved	0.98±0.19	19.1±2.2	9.85±0.84 (7)	7	—	0/7	0/7
DDT	1.84±0.21	29.1±4.0	11.84±0.95 (7)	7	0/7	—	—
DDT starved	1.36±0.19	27.5±4.2	14.80±1.68 (5)	9	—	0/9	4/9

[a] 7 days diet followed by 4 days starvation [b] 7 days diet followed by 5 days starvation. Number of animals in parenthesis is the number of animals used for activity estimation. Figures represent mean value±S.D.

complex was assayed by determination of the rate of *para*-nitroanisole *O*-demethylation[3]. Assay of total protein was performed by the Folin reaction[6]. Statistical evaluation of standard deviation and of significance (Student's *t*-test[7]) was made on programma No. 101 (Olivetti Underwood Corp.)

Results. Lindan feeding for 7 days (Table) increased the demethylation activity by 140% of the control value (significant on the level $p \leq 0.001$). After 7 days on this diet 40% of the animals were dead. DDT feeding led to a 20% increase in demethylation activity ($p \leq 0.01$) without significant shift in liver weight. Subsequent starvation of these mice caused the demethylation to increase 50% ($p \leq 0.001$) and 25% ($p \leq 0.01$), respectively, compared to normal controls and to the unstarved DDT feeding group. On the 4th day of starvation, changes in normal behaviour of mice were observed in comparison with the control starved group. These changes were characterized by uncoordinated and stereotyped movements of the front and hind legs of the animals. All animals in this group (2) survived the first 4 days of starvation but 45% died on the 5th day. In the control group of starving animals no deaths were noticed on the 5th day. Starvation of control mice led to about 43% decrease in liver weight and 37% ($p \leq 0.001$) decrease in total protein content of liver cytoplasm but did not change the specific activity of cytochrome P-450 complex. No difference in liver weights was observed due to lindan intoxication or due to DDT intoxication alone; there was only a slight decrease in the starved DDT group.

Discussion. The present data indicate that starvation does not change the specific activity and total amount of the cytochrome P-450 activity in the liver tissue, as is the case with DDT feeding alone, or followed by starvation. Thus our data support the findings of HART and FOUTS[8] who found that DDT increased hepatic drug metabolism and this increase could be evoked by starvation even 2 months after DDT feeding. The increased P-450 activity during starvation of DDT-fed animals does not seem to compensate the toxic influence of DDT which, during starvation, is mobilized from the fat tissue since the survival rate was 55% after 4 days starvation of the DDT-fed animals. After 5 days starvation, no mice in the control group had starved to death. As a lipid soluble drug DDT may act on bio-membranes, e.g. the membrane-bound cytochrome P-450 complex, explaining the high activity on this enzyme as well as on the ATP-ase as demonstrated by MATSUMURA et al[9]. The effect on the ATPase may explain the behavioural abnormalities found in the present paper and this inhibition may well be the cause of death due, for instance, to respiratory insufficiency. These findings are analogous to the demonstration of organo-chlorine poisoning following starvation of sparrows[10] and rats[11–13].

The toxicity of lindan is more pronounced than that of DDT, since in mice the activity of the P-450 complex increased 140% and the lethal effect was nearly 40% after 7 days feeding. The lethal effect was so high that the starvation period could not be survived by any animal.

Zusammenfassung. Gewicht, Gesamtprotein der Cytoplasmafraktion sowie die Aktivität des P-450-Komplexes der Leber wurden bei Mäusen nach Intoxikation mit DDT bzw. mit Lindan vor und während einer totalen Hungerperiode untersucht. Bei den hungernden Kontrolltieren war der Gesamtproteingehalt in der Cytoplasmafraktion der Leber niedrig. DDT und Lindan bewirkten eine Zunahme der Aktivität des P-450-Komplexes um 50% und 140%. Die Lindanintoxikation war stark ausgeprägt, so dass die betreffenden Tiere das Hungern nicht aushielten. Die hungernden, mit DDT vergifteten Mäuse wiesen eine weitere Steigerung der Aktivität des P-450-Komplexes auf; ihre Sterblichkeit betrug ca. 45%. Diese Tiere zeigten am Ende der Hungerperiode auch neurologische Störungen mit Einschränkungen der Bewegung.

J. CLAUSEN and G. KONAT

The Institute of Hygiene,
University of Copenhagen,
and the Neurochemical Institute,
58 Rådmandsgade, DK-2200 Copenhagen (Denmark),
19 November 1971.

[1] H. L. HARRISON, O. L. LOUCKS, J. W. MITCHELL, D. F. PARKHURST, C. R. TRACY, D. G. WATTS and V. J. YANNACONE, Science *170*, 503 (1970).
[2] J. R. GILLETTE, Adv. enzym. Regulat. *1*, 215 (1963).
[3] G. KONAT and J. CLAUSEN, Environ. Physiol. *1*, 72 (1971).
[4] E. MEZEY, J. QUARTNER and P. P. NAIR, Biochem. Med. *5*, 30 (1971).
[5] W. E. DALE, T. B. GAINES and W. J. HAYES, Toxic. appl. Pharmac. *4*, 89 (1962).
[6] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[7] F. E. CROXTON, *Elementary Statistics with Application in Medicine and the Biological Sciences* (Dover Publishers Inc., New York 1953).
[8] L. C. HART and J. C. FOUTS, Nauyn Schmiedeberg Arch. exp. Path. *249*, 486 (1965).
[9] F. MATSUMURA, T. A. BRATKOWSKI and K. C. PATIL, Envir. Contr. Toxicol. *4*, 363 (1969).
[10] R. T. BERNARD, Publ. Museum Michigan State Univ. *2*, 155 (1963).
[11] W. E. DALE, T. B. GAINES and W. J. HAYES, Toxic. appl. Pharmac. *4*, 89 (1962).
[12] J. M. BARNES and D. F. HEATH, Br. J. indust. Med. *21*, 280 (1964).
[13] D. F. HEATH and M. VANDEKAER, Br. J. indust. Med. *21*, 269 (1964).