BIOCHEMICAL MECHANISMS OF DRUG ACTION[1]

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Chlorpromazine

Effects on D-*amino acid oxidase.*—Yagi *et al.* (1) were the first to observe that D-amino acid oxidase was inhibited by low concentrations of chlorpromazine. Lasslo & Meyer (2) have examined this interaction more closely. Chlorpromazine at a 1×10^{-3} *M* concentration, caused a 40 per cent inhibition of the hog kidney enzyme, and 7.6×10^{-6} *M* FAD[4] prevented the inhibition. However, when the enzyme was preincubated with the inhibitor until the reaction between the two was complete, it was no longer readily reversible. Yagi *et al.* (3) have made further investigations on this problem. Chlorpromazine, at a high concentration was found to complex directly with FAD ($k=1\times10^{-3}$ *M*) or derivatives thereof. The phenothiazine also competed with the nucleotide for D-amino acid oxidase apoenzyme, the dissociation constants being 2.3×10^{-5} and 1×10^{-7} *M* respectively. The site of binding of the inhibitor was shown to be the same as that of the AMP part of FAD. The authors conclude that " . . . the complex formation of chlorpromazine with FAD is negligible compared with the competition of chlorpromazine with FAD [for the apoenzyme]." In another article (4) these authors investigated the effects of FAD on the EEG of a rabbit depressed by chlorpromazine. The nucleotide was found to reverse the effects of the drug. The significance of this observation is difficult to evaluate, because there was no report on the effect of FAD alone on the EEG; and, furthermore, in another article (3) the authors state that other substances (e.g. *p*-aminosalicylic acid and *p*-aminophenol) reacted with FAD and with D-amino acid oxidase apoprotein in the same manner as did chlorpromazine.

Effects of chlorpromazine on mitochondrial respiration and coupled phosphorylation.—Intensive work in this area has been conducted by two groups. Löw (5) reported in 1959 that a low concentration of chlopromazine (5×10^{-6} *M*) stimulated dinitrophenol-induced ATPase while inhibiting the mag-

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[4] Abbreviations which are used: AMP (adenosinemonophosphate); ATPase (adenosine triphosphatase); CoA (coenzyme A); DNP (2,4-dinitrophenol); FAD (flavin adeninedinucleotide); GSH (reduced glutathione); NAD [nicotinamide adenine dinucleotide (DPN)]; NADP [nicotinamide adenine dinucleotide phosphate (TPN)]; P_i (orthophosphate); ~P ("high-energy" phosphate).

nesium-induced enzyme in rat liver mitochondria respiring on glutamate When the concentration of the drug was increased to 10^{-4} *M* the DNP ATPase was depressed along with Pi^{32}-ATP exchange. This action was prevented by 2 to 4×10^{-3} *M* amobarbital (Amytal) and also by flavin nucleotides. Furthermore, quinacrine (Atabrine) had the same stimulatory effect on DNP-ATPase as did chlorpromazine. This led to the conclusion that chlorpromazine and related drugs compete with flavins, and consequently inhibit the transfer of ~P from the flavoprotein cytochrome *c* reductase to ADP. When the drug concentration was 5×10^{-5} *M* or greater no uncoupling of phosphorylation from oxidation was seen, contrary to previous work; because the transfer of electrons, as well as DNP and Mg-ATPases, were inhibited at the flavoprotein step.

Dawkins *et al.* have published a series of papers dealing with the same general problem (6). In their first publication, they described chlorpromazine as an uncoupling agent at the step of oxidation of cytochrome *c* in both liver and brain mitochondria. No such effect was seen between the substrate and cytochrome *c*, because, in agreement with Löw, oxidation and phosphorylation were inhibited to an equal degree. Finally, chlorpromazine (2×10^{-4} *M*) also inhibited cytochrome-*c* oxidase, an effect which seemed to be competitively reversed by cytochrome-*c*. Of the two effects, uncoupling of oxidative phosphorylation and inhibition of electron transport, the latter was thought likely to be of greater biological significance.

In a subsequent paper (7) Dawkins *et al.* examined more closely the action of chlorpromazine on the reduced NAD-cytochrome-*c* reductase. Like Löw they found that the drug at 10^{-4} *M* concentration inhibited the transfer of electrons at this step without uncoupling the phosphorylation. But phosphorylation was essential, since chlorpromazine was ineffective in depressing oxidation in the non phosphorylative succinate-cytochrome-*c* system. The specific site of action was thought to be on a phosphorylative step which became rate limiting in the presence of the drug, but without breakdown of the theoretical X~P, thus differing from the action of dinitrophenol. In the latest paper from this group (8) evidence was presented that 10^{-4} *M* chlorpromazine inhibited the DNP-stimulated ATPase of intact mitochondria, and the magnesium-activated enzyme in submitochondrial preparations. These results were interpreted as being a consequence of a reaction of the drug with the flavoprotein cytochrome-*c* reductase. The participation of the phosphorylated flavoprotein (X~P above) in either the transphosphorylation of ADP or the cleavage of the ~P by AT Pases would then be inhibited.

There is thus good agreement between the work of Löw and of Dawkins *et al.* However, they differ considerably in their interpretations of the oxidative phosphorylative reaction. Furthermore, at present it is difficult to judge the significance of the findings in terms of the pharmacologic actions of the promazine tranquilizers. A similar conclusion was reached by Helper *et al.* in 1958 (9). They found that 13 tranquilizers including phenothiazines, phenylcarbinols, and reserpine inhibited oxidation in brain homogenates with a

maximum effect on cytochrome oxidase at 1×10^{-4} *M* (chlorpromazine). The significance of these effects in terms of the mechanism of action of the drugs was considered doubtful, because of their low potency, lack of specificity, and the fact that greater inhibitions were seen with liver than with brain homogenates.

Grossi *et al.* (10) have examined the incorporation of a number of labeled substrates into carbon dioxide, cholesterol, acetone-soluble fatty acids, and phospholipid fatty acids of rat brain slices when chlorpromazine was either added *in vitro* or administered *in vivo* (3.5 mg/kg). The most consistent effect seen was a stimulation of incorporation of the substrates into phospholipids in the presence of 10^{-5} *M* drug *in vitro*, or after injection of chlorpromazine into the animals. An *in vitro* concentration of 10^{-3} *M* depressed the incorporation. The authors concluded, that the effects of the drug on brain phospholipid synthesis provided new evidence for the specific pattern of fatty acid synthesis in the brain. This statement, rather than any interpretation regarding the mechanism of action of chlorpromazine, was in keeping with the finding that the above results were demonstrable in growing rats only.

Weiner & Hŭls' (11) have made some observations, the significance of which should be noted by all workers who study the action of drugs on brain metabolism. If rats were sacrificed by decapitation, and the head dropped directly into liquid oxygen, pretreatment with chlorpromazine in a wide range of doses up to 800 mg/kg was shown to result in higher brain concentrations for ATP and creatine phosphate, and lower AMP than was seen with controls. However, when the whole animal was frozen, no demonstrable changes in the nucleotides occurred. Creatine phosphokinase and nucleotide degrading enzymes were not affected by drug treatment. While the higher levels of nucleotides that were found in decapitated chlorpromazine treated animals might reflect inhibition of ATPase, it is more likely that they reflect the depression by the drug of the stimulation which follows decapitation.

Wollemann & Elodi (12) have described an interesting effect of phenothiazine derivatives, and some aminoketones on glyceraldehydephosphate dehydrogenase. When chlorpromazine was incubated at a concentration of 10^{-6} to 10^{-5} *M* with the enzyme, inhibition of the latter took place, but only in the presence of light. Nor was inhibition produced in the dark by chlorpromazine after the drug had been preincubated in light. Cysteine prevented the action of the drug, but this was not simply a result of protection of enzyme sulfhydryl groups. Incubation of the drug with the enzyme alone resulted in an alteration in the absorption spectrum of the former which was prevented by cysteine. These and other data are interpreted as indicating that glyceraldehydephosphate dehydrogenase catalyzes an oxidation of the drug. The product is thought not to be chlorpromazine sulfoxide, on the basis of the absorption spectrum, and the fact that this derivative is much less of an enzyme inhibitor than the parent drug.

BARBITURATES

Aldridge & Parker (13) have reinvestigated the effect of six barbiturates on oxidative-phosphorylation in liver mitochondrial preparations. They have confirmed earlier findings (14) that thiobarbiturates are uncoupling agents, but state that oxybarbiturates are not. Their data for hexobarbital and phenobarbital are very similar to those of Brody & Bain (14), who found these two oxybarbiturates to be a weak and a non-effective uncoupler, respectively. However, Aldridge & Parker with the other oxybarbiturate they studied (amobarbital) found no uncoupling over the concentration range 0.06–0.5 *mM;* whereas, Brody & Bain found a 46 per cent depression of oxidative-phosphorylation at 0.3 *mM*. The reason for this difference is not immediately apparent, although the experimental systems differ in some details, and Aldridge & Parker worked exclusively with liver mitochondria. They also report that the barbiturates stimulate mitochondrial ATPase, and give values for the magnitude of this effect which are essentially the same as those found by Bain (15). They do not attribute much significance to the twofold increase produced by, amobarbital. Cohen & Heald (16) have found that phenobarbital does not affect the rate of resynthesis of phosphocreatine in electrically stimulated brain slices. In harmony with its ability to suppress the increased oxygen uptake brought about by stimulation, phenobarbital also protected the slices against the phosphocreatine depletion which is the usual concomitant of such stimulation. It should be noted here that another variable has been discovered in such experimental arrangements, in that the metabolic response to stimuli, and the effect of drugs have been found to vary with the predominance of white or grey matter in the tissue section, and even with the orientation of the fibers with respect to the application of the stimuli (Kurokawa, 17).

Several other studies on *in vitro* preparations have appeared (18, 19, 20) dealing with the effects of barbiturates and other depressants upon oxygen and phosphate metabolism. Similarly, a number of *in vivo* studies have also been reported (21, 22, 23, 24, 25). Such data, while interesting, have not provided new insight into the fundamental mechanism(s) which may be responsible for their depressant action. Some mechanism, such as that suggested by Skou (26) who has evolved an hypothesis relating ATPase and the transport of sodium and potassium across the nerve membrane, may yet be found to provide the link between phosphate metabolism, and the action of depressants on nervous function. Jarnefelt's (27) findings bearing on this hypothesis, are that rat brain ATPase activity could be increased by sodium ion, and that this increase could be blocked by pyridine aldoximedodecyliodide at the relatively low concentration of 3×10^{-5} *M*. The effect on the sodium ion stimulation was specific for the drug, related detergents and alcohols inhibiting both stimulated and nonstimulated enzyme activity.

The pharmacologic significance of the known biochemical effects of the CNS depressants has been reviewed a number of times in the last few years, the most recent discussion being that of Heald (28). As yet, none of these bio-

chemical effects either singly, or in concert, provide an adequate explanation for the pharmacological depressions. We can only close this discussion by calling attention to an intriguing new suggestion by Pauling (29), that formation of minute hydrate crystals of the clathrate type in tissues might be an explanation for the depressant action of general anesthetics.

Ethanol

Interest has been revived in the classical depressant, ethanol, since its actions were last reviewed by McIlwain (30). While high concentrations (0.1 *M*) are required, it is of interest that inhibition of respiration of electrically stimulated brain cortex slices can be demonstrated; whereas, as shown by Beer & Quastel (31) with potassium stimulated slices, unstimulated slices are not affected (Wallgren, 32). Another *in vitro* effect is the stimulation by ethanol of the formation of fatty acids from acetate by liver slices persumably by increasing the level of reduced NAD [Lieber & Schmid, (33)]. *In vivo* a dose of 2 g/kg in the rabbit produces an acute depression of serotonin and norepinephrine levels of the brain stem which persists long after the ethanol has disappeared. Chronic administration reduces the levels of these amines in the brain stem by 50 per cent [Gursey & Olson, (34)].

Salicylates

The action of salicylates on metabolism was reviewed by M. J. H. Smith (35) two years ago, and many of the papers on this subject appearing since then have come from his group. The relation between chemical structure and uncoupling effect on the process of oxidative-phosphorylation has been restudied by Brostoff *et al.* (36) using a yeast test system which gives somewhat different results than those of mammalian test systems. For example, salicylamide which has been reported by two different groups of investigators to be inactive in mammalian mitochondrial systems (35), was found to be active in the yeast system. A series of reports have appeared on the incorporation by several mammalian tissues of C^{14} from labeled glucose, pyruvate, glutamate, and acetate into a variety of metabolic intermediates both *in vitro* and *in vivo*, as affected by salicylate and 2,4-dinitrophenol. They are interpreted by the authors as being consistent with the uncoupling activity of these two agents plus, in the case of salicylate, interference with transamination reactions (37, 38, 39, 40, 41). Because pool sizes and specific activities were not determined even this general interpretation is subject to qualification. Other reports continue to appear testifying to the stimulatory effects of salicylates on glucose utilization, glycogenolysis, and intermediary carbohydrate metabolism in liver, brain, and muscle (42, 43, 44, 45). All of these may be attributed to a loss of regulation via the Pasteur mechanism as a result of uncoupling of the oxidative-phosphorylation reactions, but other explanations are also possible.

Sproull (46) found that salicylate, benzoate, and gentisate increased the reduced glutathione level in livers from male but not female rats; whereas,

antipyrine produced the effect in females, but not in males. Other anti-inflam matory drugs, phenylbutazone, chloroquin, cortizol, and dexamethasone were studied for their effect on incorporation of C^{14} from labeled glucose, acetate, and pyruvate by rat liver and brain preparations, and were found to have little in common with salicylate except a reduction of label incorporation into alanine from glucose by the liver preparations [Moses & Smith (47)]. Uncoupling of oxidative-phosphorylation appears to have little connection with anti-inflammatory activity since Marks, Smith & Cunliffe (48) showed that DNP had little effect on dye leakage from capillaries during anaphylaxis in the guinea pig, a test system which is remarkably responsive to the salicylates. Serum transaminase levels are decreased by salicylate (39), but are increased by aminopyrine [Fazekas, *et al.* (49, 50)]. Allyloxybenzamide, an antipyretic salicylamide derivative, has been reported not to uncouple oxidative-phosphorylation although it did inhibit cytochrome chain oxidations in rat liver mitochondria [Bruni & Contessa (51)]. Simple note of these conflicting observations makes it obvious that no unifying biochemical observation has yet appeared as an explanation for the diverse effects of the salicylates and other drugs with like actions.

Monoamine Oxidase Inhibitors

The subject of monoamine oxidase inhibitors was extensively reviewed last year by Burns & Shore (52) and Rosenblum & Ferguson (53). Numerous papers on this general subject have appeared since then, but limitations of space will restrict our coverage to selected papers which report actual measurements of enzyme activity. Gey & Pletscher (54) found that 85 per cent of the monoamine oxidase activity of the brain must be inhibited before an appreciable rise in 5-hydroxytryptamine or norepinephrine can be observed. They conclude that the function of this excess of enzyme is to rapidly inactivate free monoamines rather than to regulate the total amine content. The analogy to the cholinesterase system is obvious. Considering this large excess of enzyme, the failure of Schuler & Wyss (55) to demonstrate unambiguous correlation between monoamine oxidase inhibitory potency and reserpine antagonism, requires clarification before this antagonism may be attributed solely to amine accumulation. However, a good correlation between the duration of the behavioral effect in pigeons of 5-hydroxytryptophan, and the recovery of brain monoamine oxidase activity after inhibition by iproniazid, was observed by Aprison & Ferster (56).

On the basis of inhibition experiments with trans-2-phenylcyclopropylamine Green & Erickson (57) estimate that the turnover of norepinephrine in the brain is about 17 per cent per hr, this figure resting on the assumption that monoamine oxidase is the major route of metabolism in the brain. Two studies have appeared supporting the contention that the monamine oxidase-inhibiting activity of iproniazid *in vivo* is the effect of its catabolite, isopropylhydrazine, and not the parent molecule (58, 59). Weiss-

bach *et al.* (60) have shown that there are alternate pathways for the metabolism of 5-hydroxytryptamine, the major one leading to the formation of 5-hydroxytryptamine-O-glucuronide, which become apparent when monoamine oxidase is inhibited. An analogous situation evidently exists for epinephrine, because after blockade of both monoamine oxidase and catechol-O-methyltransferase a new epinephrine catabolite and increased production of acidic, mainly conjugated catabolites, was observed [de Schaepdryver & Kirshner (61)].

Crout (62) has reported some basic data on the inhibition kinetics of catechol-O-methyltransferase by pyrogallol. The enzyme isolated from rat liver, brain, or heart was found to have the same K_m for norepinephrine (3×10^{-4}) and for S-adenosylmethionine (4×10^{-5}) regardless of source. Inhibition by pyrogallol was found to be partially non competitive with a K_i of 8×10^{-6} for the liver enzyme. These findings are somewhat different from those of Axelrod *et al.* (63) who reported different K_m for liver and brain enzyme and inhibition of a competitive type; but since the earlier workers used epinephrine as a substrate, an unambiguous comparison is not possible.

γ-Aminobutyric Acid, Vitamin B_6, and Hydrazides

Numerous papers have appeared over the past year on the subject of γ-aminobutyric acid, vitamin B_6, and their relationship to seizure production which were reviewed by Tower (64), Burns & Shore (52), Roberts & Eidelberg (65) and Williams & Bain (66). There has been an accumulation of reports which emphasize the opinion that there is no direct and simple relationship between seizure susceptibility and the level of γ-aminobutyric acid in the brain (67, 68, 69, 70, 71, 72). Even though there is no question that the amino acid has inhibitory effects on a variety of neural structures, the correlation between the actual level of γ-aminobutyric acid and seizure susceptibility is so capricious that under certain conditions no unifying generalization is yet possible. Convulsants like metrazol have long been known to produce seizures in the presence of normal levels of γ-aminobutyric acid, but aminooxyacetic acid [Wallach (73)], or a combination of thiosemicarbazide and hydroxylamine (69), may produce a rise in the levels of γ-aminobutyric acid and convulsions simultaneously. Some of the confusion may arise from failure to distinguish between free and bound forms of the amino acid. Elliott & Van Gelder (74) have shown, for example, that the rise in γ-aminobutyric acid produced by hydroxylamine is mostly in the free form, whereas, the fall produced by hydrazides is a reduction in the bound form. *In vitro* studies have shown that the uptake and production of the amino acid by brain cortex slices is a function of the ionic composition of the medium [Rybova (75)]; and, in addition, that it may accumulate in slices against a concentration gradient in a process requiring energy and the participation of pyridoxal [Tsukada *et al.* (76)], or pyridoxal phosphate [McKhann *et al.* (77)]. In work with brain mitochondria, McKhann & Tower

(78) have reemphasized the reciprocal relationship between alternate pathways of α-ketoglutarate breakdown for the regulation of γ-aminobutyric aicd production.

A discussion of the neurophysiology involved is beyond the scope of this review, but the authors should like to emphasis the statement of Roberts & Eidelberg (65) that " . . . the failure of the neurobiochemist and the neurophysiologist to understand anything but the most superficial aspects of the companion science . . . " is one of the chief difficulties interposed between the finding of correlations, and the establishment of causal relationships between chemically and electrically observed events.

Just as the details of action of γ-aminobutyric acid are still being accumulated, so reports bearing on the originally observed relationship between hydrazide-produced seizures, and vitamin B_6 [for review see (66)] continue to appear (72, 79, 80, 81, 82, 83, 84). Snell and his collaborators (83, 84, 85) have extended their work on the pyridoxal phosphokinases. They have reported that the kinases are found in all tissues with the highest levels in liver, kidney, and brain, and that pyridoxal, pyridoxine, and pyridoxamine are all phosphorylated (85). Of interest here is that carbonyl reagents, such as, hydrazine, hydroxylamine, and isoniazid inhibit purified preparations of the kinase at 1/100th to 1/1000th the concentration required to inhibit glutamic decarboxylase (85). Further, in confirmation of their earlier report (86), condensation products of pyridoxal and hydroxylamine, *O*-substituted hydroxylamines, hydrazine, and substituted hydrazines were found to be extremely potent kinase inhibitors, and interestingly enough, the brain kinase is more sensitive than the liver kinase. The hydrazones of pyridoxal phosphate were not inhibitors of the kinase, nor were such agents as barbituates, succinimides, or diphenylhydantoin. These data may be taken in support of their earlier suggestion, that the hydrazides act to lower GABA levels by an indirect mechanism in which the inhibition of the production of pyridoxal phosphate is the primary event, rather than by elimination of pyridoxal phosphate as a coenzyme through hydrazone formation (66), or direct inhibition of glutamic decarboxylase.

Orally Active Hypoglycemic Drugs

This subject has been covered in three recent reviews (87, 88, 89) which have summarized the evidence leading to the conclusion that the sulfonylureas produce their hypoglycemic effect by stimulating the release of endogenous insulin. Admittedly there are still anomalous results which are not well explained by this hypothesis. Similarly, the currently accepted but by no means satisfactory view is that the biguanides act by inhibition of the areobic electron transport system with consequent loss of the Pasteur effect. A few studies bearing on mechanism of action have appeared since the coverage of the above reviews. Schumacher (90) using chlorpropamide has added another report to those who find a positive effect on inorganic phosphate uptake from the blood by the sulfonylureas. Shelley *et al.* (91) using hepta-

tectomized and porto-caval shunt animals, and Leonards *et al.* (92) using indwelling catheters and isotopic glucose, have extended the already substantial data indicating that release of endogenous insulin is the prime mode of action of tolbutamide. Further, these two groups of investigators also present data to indicate that tolbutamide induces increased peripheral utilization of glucose. In the case of the experiments of Leonards *et al.* (92), the data were interpreted as showing that this occurred even in the presence of the liver; whereas, Shelley *et al.* (91) could not demonstrate increased peripheral utilization of glucose except in hepatectomized animals.

Gryglewski & Duncan (93, 94) have reported that tolbutamide decreases GSH blood levels in non-diabetics and in responsive diabetics, but not in resistant diabetics. Insulin, even though it produced equivalent blood sugar reductions had no effect on GSH levels. Essentially, the same results were obtained in rats; and, in addition, tolbutamide when perfused through the liver *in situ* produced a fall in tissue GSH which was not duplicated by insulin or sulfadiazine. Interpretation of these results, based on the premise that tolbutamide action is not entirely explained by insulin release, is attempted by suggesting that reducing GSH levels may remove inhibition of pyruvic dehydrogenase in liver, and accelerate the utilization of glucose, or depress the activity of the liver enzyme which reduces insulin with GSH as a coenzyme. [Williams (95)]. Along this same general line, Middleton & McLaughlan (96) have shown that tolbutamide and chlorpropamide hypoglycemia are reduced in pantothenic acid deficiency, which has no effect on insulin or phenformin hypoglycemia. They also showed that tolbutamide reduced the ability of the normal rat to acetylate sulfanilamide, so that evidence continues to accumulate showing that there are several potential sites at which the sulfonylureas may affect carbohydrate metabolism. The relative importance of these various possibilities cannot yet be assessed.

Biochemical Effects of Catecholamines and Adrenergic Blocking Agents

In the past two years we have collected several hundred references dealing with the biochemical actions of catecholamines and their inhibition by adrenergic blockade. Fortunately, several excellent reviews have appeared during this time, to which we refer the reader. Stetten & Stetten (97) have examined this subject from the point of view of glycogen metabolism. Ellis (98) has reviewed the relation of the biochemical effects of epinephrine to its action on muscle. Tepperman & Tepperman (99) have examined the catecholamines as part of their survey of hormones. Sutherland & Rall (100, 101, 102) have reviewed the relation of adenosine-3′, 5′-phosphate to the action of catecholamines. Finally, Lundholm & Mohme-Lundholm (103) have summarized the action of catecholamines on carbohydrate metabolism in smooth and skeletal muscle.

The action of catecholamines on smooth muscle.—Axelsson & Bülbring

(104) and Axelsson *et al.* (105) have examined the hypothesis that inhibition of electrical activity, and of tone of intestinal smooth muscle, are controlled by metabolic factors, which in turn are stimulated by epinephrine. In support of this hypothesis, it was found that alteration of metabolic rate by change in temperature, glucose addition and withdrawal, metabolic inhibitors, and substitution of lithium for sodium in the medium in which guinea pig teneae coli were suspended, produced changes in electrical activity and tension. These changes showed that stimulation of metabolic rate inhibited spontaneous action potentials, electrical excitability, and tension, i.e. the increased energy supply was used to stabilize the smooth muscle cell membrane (104). Epinephrine had the same effect as an increase in metabolic rate, and, furthermore, it increased the activity of glycogen phosphorylase. The abolition of the response to epinephrine when lithium was substituted for sodium suggested that the end result of increased metabolism was to augment the active extrusion of sodium from the muscle cells (105). However, a direct link between epinephrine-stimulated glycogenolysis, or increased carbohydrate metabolism in general, and active sodium extrusion remains to be proven. Furthermore, the report of Axelsson *et al.* (105) shows that the correlation between the electrophysiological effects of epinephrine and its action on phosphorylase is by no means clear.

In contrast to intestinal smooth muscle, decreased tone of vascular smooth muscle was associated with a decrease in energy metabolism [Lundholm & Mohme-Lundholm (106)]. When metabolism was stimulated, tone also increased. However, both barium and histamine could augment the latter without an increase in energy production, so that here, too, additional efforts are needed before a definite relationship between the effects of these drugs on energy metabolism can be directly related to their physiological actions.

Catecholamines and adrenergic blocking agents on skeletal muscle and heart.—The basic problems in the relation between energy metabolism and muscular contraction have recently been reviewed by Mommaerts *et al.* (107). One aspect which deserves emphasis is the effect of the incubation medium and the process of preparation on the metabolic response of an isolated rat diaphragm to epinephrine. Herman & Ramey (108) have shown that the ability of the amine to inhibit glucose uptake was a function of the concentration of magnesium and phosphate perhaps acting on the rate limiting enzyme of glycolysis, phosphofructokinase. Bouman & Dermer (109, 110) have pointed out that the initial level of glycogen in the diaphragm *in vitro* has important effects on the subsequent action of epinephrine. Thus, if the diaphragm was removed from a rat which had been decapitated without anesthesia, initial glycogen was much lower than if the animal had been pretreated with pentobarbital. In the former case epinephrine merely slowed resynthesis of the polysaccharide, but in the latter there was definite acceleration of glycogenolysis.

The relation between the activation of myocardial phosphorylase by

drugs and their inotropic action on the heart was reviewed by Cotten & Moran in 1960 (111). Recently Haugaard *et al.* (112) have demonstrated that the high values which had been found for phosphorylase *a* (the active form of the enzyme) in control perfused rat hearts were due to partial activation by AMP. If the nucleotide was removed from the heart extract with an anion exchange resin, it was then observed that 80 to 90 per cent of the enzyme was in the *b* or inactive form. This is significant for two reasons. It indicates that in the unstimulated heart there is little active phosphorylase, and secondly, it permits the demonstration of augmentation of enzyme activity with greater sensitivity than was previously possible. LaCroix & Leusen (113) have reported that the cardiac adrenergic blocking drug, dichloroisoproterenol (DCI) did not prevent the epinephrine-induced augmentation of phosphorylase *a* in isolated rat hearts. However, their control values are very high and the DCI itself increased activity of the enzyme. It is difficult to compare these results to the finding by Mayer & Moran (114) that DCI blocked the activation of phosphorylase in the intact dog heart.

Hess *et al.* (115) have reported that blockade of sympathetic activity in the intact rat with hexamethonium, bretylium, and reserpine lowered the phosphorylase *a* content of myocardium. Anesthesia had the same effect, probably because it ameliorated the shock of decapitation [see also (11)]. This method of sacrifice was associated with high control values for enzyme activity, despite the use of anion exchange resin. It would be of interest to determine if sympathetic blockade would reduce phosphorylase *a* if control values were as low as those reported in the earlier paper from this group (112). Another possibility to consider is the effect of the blocking agents which were used on blood pressure and coronary flow. Klarwein *et al.* (116) have recently shown that the alterations in active phosphorylase which occurred in the intact dog heart during tachycardia and fibrillation were abolished when coronary circulation was maintained. Belford & Feinleib (117) have found that reserpine treatment for one to seven days significantly reduced the per cent of phosphorylase *a* in the heart of intact rats and in some cases also the cerebral cortex. Control values were high, and one is again reminded of the work of Weiner & Huls (11). However, Belford & Feinleib also found that other central nervous system depressants—chlorpromazine and pentobarbital—had no effect on the enzyme.

Other interesting papers on metabolic aspects of adrenergic control in muscle are those of Schwartz & Lee (118), and Sréter & Friedman (119). The former investigators discovered that one day after a dose of 5 mg/kg of reserpine, cat and guinea pig ventricles were almost devoid of catecholamines, and there was a marked uncoupling of oxidative phosphorylation in mitochondria prepared from these hearts. However, no alteration of ATP, creatine phosphate and Pi were seen. This suggested that either the degree of uncoupling was insufficient to alter the concentrations of these compounds, or that this was somehow related to the decreased work done by the reserpinized heart. Sréter & Friedman (119) have described experiments with skeletal

muscle *in situ* and *in vitro* in which phenoxybenzamine (Dibenzyline) diminished the contractile response and uptake of sodium proportional to potassium loss when the muscle was stimulated through its nerve for 1 hr. Thus, alterations in autonomic mechanisms which appear to be related to muscle contraction can manifest themselves in a variety of phenomena, none of which have yet been shown to be of primary significance.

Other metabolic aspects of adrenergic blockade.—A variety of processes and chemicals have now been shown to interfere with the metabolic alterations attributed to the catecholamines. Ether anesthesia potentiated some, and depressed other responses of patients to epinephrine and insulin [Henneman & Vandam (120)]. High altitude hypoxia has been reported to result in increases of several enzymes in the serum of dogs. This response was diminished by prior administration of phenoxybenzamine (Dibenzyline) [Highman & Altland (121)]. The cardiac adrenergic blocking drug, dichloroisoproterenol, has been shown by Mayer *et al.* (122) to block the hyperglycemia and hyperlactic acidemia in response to catecholamines. Ergotamine did not interfere with the increase in blood lactate. Claassen & Noach (123) found that dichloroisoproterenol did not block the hyperglycemic action of norepinephrine in rats. This was said to agree with the classification of catecholamines in terms of their reaction with receptors, but also indicates that there can be species differences in the action of adrenergic blocking drugs. Thus, there have been reports of the effectiveness of β-haloalkylamines in preventing epinephrine-induced hyperglycemia [see Nickerson (124)], but Mayer *et al.* (122) did not find this in the dog with a 15 mg/kg dose of phenoxybenzamine. Perhaps the attempt to fit the metabolic responses to catecholamines into a simple scheme of receptors is premature.

The hyperglycemia accompanying hypothermia in dogs is apparently an adrenergically induced response. Kilburn (125) has shown that it was not due to shivering, nor to a decrease in renal excretion of glucose, but was abolished by 4 mg/kg of a mixture of hydrogenerated ergot alkaloids (Hydergyn). The defect in temperature control of baby rabbits when exposed to 42 to 45 ambient temperatures was associated with a rise in blood glucose which was abolished by the ganglionic blocking drug pentolinium and by ergotamine [Chaudhuri & Sadhu (126)].

The literature on the action of catecholamines on lipid metabolism, specifically the release of fatty acids from adipose tissue lipids, is extensive and of great interest. However, limitations of space and time do not permit a careful review. There have been many reports of interference with the release of free fatty acids from fat depots both *in vivo* and *in vitro* by adrenergic blocking drugs. Among the most recent is the report of Schotz & Page (127) that phentolamine, phenoxybenzamine, and N-(2-chloroethyl)dibenzylamine (Dibenamine) blocked the epinephrine-induced release of free fatty acids from the epididymal fat pad of rats, when the blocking drugs were given *in vivo*. Dichloroisoproterenol had a similar effect on the rise of free fatty acids in plasma following the administration of catecholamines to dogs (122).

Anticoagulants

The well known uncoupling effect of bishydroxycoumarin (Dicumarol), plus the recent interest in the role of quinone cofactors in oxidative phosphorylation, has stimulated some excellent work on the biochemical effects of anticoagulants. Ernster *et al.* (128) have purified a soluble liver diaphorase which catalyzes the transfer of electrons between reduced NAD or NADP and a variety of electron acceptors including vitamin K_3. The enzyme was inhibited 50 per cent by dicumarol (10^{-8} *M*), thyroxin and triiodothyronine (6×10^{-5}) and the flavin antagonists quinacrine and chlorpromazine (10^{-3} *M*). The inhibition by bishydroxycoumarin was independent of the concentration of vitamin K_3, and occurred when other electron acceptors were used. Wosilait (129) has described an enzyme from dog liver which may be the same as the one used by the Swedish group. Using a solubilized preparation of vitamin K_1 as the electron acceptor from reduced NAD and NADP, Wosilait found it to be inhibited 38 per cent by 10^{-6} *M* bishydroxycourmarin (129). It should be noted that Ernster *et al.* found that vitamin K_3 was a much better electron acceptor than was K_1. Wosilait has also examined the reduction of coenzyme Q_{10} with his enzyme in the presence of a variety of anticoagulants. Bishydroxycoumarin inhibited the enzyme 59 per cent at 10^{-5} *M*. The other drugs tested, cumachlor, ethyl biscoumacetate (Tromexan), warfarin, phenindione, and acenocoumarin (Sintrom), were effective at 10^{-4} *M* concentration or higher.

In another investigation, Wosilait (130) pointed out that anticoagulant potency and inhibition of vitamin K_1 reductase, paralleled reduction of rat liver slice respiration. Bishydroxycoumarin (7.5×10^{-5} *M*) produced a 50 per cent inhibition of oxygen consumption in the latter preparation in one hour. Inhibition continued to increase after this time, but drug uptake by cell fractions became maximal in 75 minutes. The maximum amount bound was in the range of concentrations at which the drug is found in the liver after administration of effective amounts to intact animals. Various nitrophenols lowered oxygen consumption, and inhibited purified vitamin K_1 reductase. Dinitrophenol was the most potent at 5×10^{-4} *M*.

Wosilait (131) concludes that these observations do not permit any causal relation to be established between the effects of anticoagulants on respiration and their action on electron transport. It might be added that the same applies to the biochemical effects of these drugs and their thereapeutic action.

Williams (132) has demonstrated that phenylinandione is a competitive antagonist of the stimulant action of menadione on iodine binding by thyroid slices. Since other workers have shown that the action of menadione was not blocked by antimycin A or amobarbital, the interaction of the vitamin and the anticoagulant is not thought to be concerned with electron transport, but perhaps with the iodination of tyrosine. As was discussed above, Ernster *et al.* (128) have demonstrated that thyroxine and triiodotyrosine inhibited a diaphorase which transferred electrons to quinones.

Carbonic Anhydrase Inhibitors

Kinetics of carbonic anhydrase inhibition.—Maren *et al.* (133) have studied the relation between structure and activity of eight sulfonamides as inhibitors of dog red cell carbonic anhydrase. An absolute requirement was an unsubstituted sulfonamide group. Compounds with the structure aryl-SO_2NH_2 had no other action on electrolyte metabolism except inhibition of the enzyme, while those with condensed heterocyclic rings (e.g. chlorothiazide) did have other actions. The K_i of the eight compounds ranged from 2×10^{-5} *M* (hydrochlorothiazide) to 1×10^{-9} (ethoxyzolamide). All inhibitors acted non competively, but reversibly. In another report Maren *et al.* (134) examined the binding of aromatic sulfonamides to erythrocytes *in vitro*. This process occurred against a concentration gradient, but was independent of pH, temperature, and metabolism. Compounds substituted on the sulfonamide group lacked affinity for the red cells, and did not inhibit the enzyme. For those agents which did inhibit carbonic anhydrase there was a good correlation between the K_i and the affinity of the drugs for the cells. Aryl sulfonamides were easily washed out, but acetazolamide, and other heterocyclic inhibitors were not. A detailed investigation of the interaction between acetazolamide or benzthiazide with carbonic anhydrase was made by Leibman *et al.* (135). The former agent was found to equilibrate rapidly with the enzyme, and the reaction was noncompetitive and reversible. Benzthiazide was a less active inhibitor, and equilibrated slowly with the enzyme. The increase in potency which occurred during the equilibration was prevented by the addition of the substrate, carbon dioxide. Finally, this drug was not clearly either a reversible or irreversible inhibitor.

Physiological correlations of carbonic anhydrase inhibition.—Wistrand *et al.* (136, 137) have examined the relation between carbonic anhydrase inhibition, and the effects of the inhibitors on the pressure and composition of cerebrospinal fluid and aqueous humor. Immediately after the administration of the drugs to dogs there occurred an increase in the pressure of cerebrospinal fluid. This was due to a sudden increase in the intracranial vascular bed resulting from the rise in peripheral venous carbon dioxide. The pressure returned to normal in one hour, and it remained there despite continued inhibition of carbonic anhydrase, and the accumulation of bound inhibitors in plasma and red cells. No alterations in cerebrospinal fluid electrolytes or carbon dioxide were found. The intra ocular pressure of dogs did fall after intravenous administration of the inhibitors, and it remained at a low level. This was associated with continued inhibition of the enzyme in the ciliary processes, and a high concentration of the drugs at these sites. No consistent changes in aqueous humor electrolytes or carbon dioxide were noted.

Erulkar & Maren (138) have reported that the carbonic anhydrase content of the cochlea was the highest yet found in any tissue. Furthermore, there was a variability in the distribution of the enzyme which appeared to be related to specialization for secretory activity. The concentration of acetazolamide in the inner ear was similar to that of plasma, but this was

sufficient to inhibit 99.9 per cent of the enzyme, and was correlated with an inhibition of potassium secretion.

The action of acetazolamide on the activity of rat kidney phosphate-activated glutaminase has been investigated by Beaton (139). After 100 mg/kg of the drug was administered to the intact animal, there was a biphasic alteration in the enzyme: first depression, followed by increased activity 16 to 24 hours after administration. The net effect on ammonia excretion was to increase it. Inhibition of the glutaminase *in vitro* appeared to be competitive, but data on kinetics are otherwise lacking. Siegmund & Dulce (140), Dulce & Siegmund (141) and Dulce *et al.* (142) have proposed that carbonic anhydrase of osteocytes is essential for the secretion of hydrogen ion, and consequent demineralization of bone. The evidence they present is: (*a*) The administration of acetazolamide to hens reduced plasma calcium, and its content in egg shell; acidosis per se did not alter the latter. (*b*) Acetazolamide was a poor inhibitor of bone phosphatase *in vitro*. It did not inhibit estrogen production in the hen, and it antagonized the effect of these hormones in increasing plasma calcium in the cock. Nor, was the site of action of the drug the shell forming gland, since acetazolamide lowered the plasma calcium of roosters also. (*c*) Considerable carbonic anhydrase activity was found in osteocytes prepared from bird and fish bones, but none was found in unossified cartilage. Koch & Woodbury (143) have found that acetazolamide reduced the rate of turnover of radiosodium in the brain, and decreased the intracellular sodium concentration. They interpret these findings as indicating a reduction in the permeability of the neuronal membrane to sodium ion, and consider this to be a possible mechanism for the anticonvulsant action of this agent.

The Cytochemical Effects of Drugs and Poisons in the Liver

This portion of the review will be concerned with the action of carbon tetrachloride, the toxins of *Amanita phalloides*, the pyrrolizidine alkaloids, and certain drugs on the function of the components of the liver cell. The action of chlorpromazine on mitochondrial metabolism has been considered separately.

Carbon tetrachloride.—Early important papers on the biochemical effects of carbon tetrachloride were those of Christie & Judah (144), and Dianzani (145) in 1954. The various parameters which were measured by these investigators suggested that the poison produced a derangement in the function of liver mitochondria. They demonstrated defects in the oxidation of fatty acids, and citric acid cycle intermediates, uncoupling of oxidative phosphorylation, and Christie & Judah found a decrease in NAD dependent dehydrogenases which was reversed by the addition of NAD *in vitro*. This indicated to these authors that the primary action of carbon tetrachloride was to produce an increase in the permeability of mitochondria. In 1961 most workers in the field agree that this is not correct, because alterations in the metabolism of liver cells can be demonstrated within a few hours after ad-

ministration of the poison without changes in mitochondrial function. However as to the specific locus of action of carbon tetrachloride there is considerable controversy.

Brody and his collaborators [Calvert & Brody (146), Moore & Brody (147), Brody & Calvert (148), and Brody *et al.* (149)] have proposed that the primary site of action of carbon tetrachloride is on the central nervous system so as to produce a sympathetic discharge. This is thought to result in constriction of the blood vessels supplying the liver and consequent anoxia. Evidence in support of this hypothesis is: (*a*) Adrenergic blockade with ergotamine and phenoxybenzamine, pretreatment with reserpine, adrenalectomy and section of the lower cervical cord were effective in varying degrees in preventing the biochemical consequence of 4 g/kg[5] orally administered carbon tetrachloride in the rat. Chordotomy was the only method which completely blocked the effects of the poison. The parameters which were measured 20 hr after administration of carbon tetrachloride were oxidative phosphorylation, activation of magnesium-ATPase in liver mitochondria, and hepatic fat deposition (146). (*b*) Ligation of the mesenteric and celiac arteries, and of the portal vein of the rat was used to produce liver anoxia. Two hours later mitochondria prepared from these livers showed changes similar to those demonstrated by these and other workers to be the consequences of carbon tetrachloride poisoning (147). (*c*) Four g/kg of the poison administered in a single dose effected a one third reduction in the catecholamine content of the rat adrenal, and a two thirds fall in the adrenal of the rabbit. This depletion was entirely prevented by prior cord section (148). (*d*) Spinal section protected completely and adrenergic blockade partly against both the biochemical and histopathological changes which occurred as early as 5 hr after administration of carbon tetrachloride. These studies demonstrated that an increase in hepatic lipids and microscopic fatty infiltration were demonstrable about 15 hr before alterations in mitochondrial metabolism (e.g. activation of magnesium ATPase), and centrilobular necrosis occurred (148). This is in agreement with other workers who have emphasized the significance of the early biochemical alterations in carbon tetrachloride poisoning.

The cytochemical alterations in liver cells which occur very soon after the administration of carbon tetrachloride have been investigated chiefly by Recknagel and his colleagues. They have proposed that its primary site of action is on the liver triglyceride secretory mechanism, inhibition of which results in the accumulation of fat. This is based on the following evidence. (*a*) After oral administration of 4 g/kg of the poison, the peak concentration of carbon tetrachloride was reached in rat liver in one to two hours. This corresponded approximately with the time of maximum accumulation of hepatic tryglycerides. No alteration in mitochrondrial function, including

[5] Where doses of carbon tetrachloride were given in ml. they were recalculated as g. with the specific gravity of the poison taken as 1.6.

fatty acid oxidation, was found during the first several hours [Recknagel & Litteria (150), Schotz & Recknagel (151), Recknagel & Anthony (152), Share & Recknagel (153), and Recknagel & Lombardi (154)]. (*b*) Microsomal enzyme systems were, however, altered during the early phase of poisoning: glucose-6-phosphatase was depressed, and reduced NAD-cytochrome *c* reductase of liver microsomes was elevated (154, 155). (*c*) The elevation of plasma triglycerides consequent to the intravenous administration of Triton was blocked by previous administration of carbon tetrachloride, and the net result was an increase in liver fat (155). (*d*) Direct evidence against the hypothesis that its primary action is on mitochondrial permeability (144, 145) was obtained (154). If carbon tetrachloride was added to liver mitochondria prepared from rats which had been poisoned with fluoroacetate, the high concentration of citrate in mitochondria, resulting from the latter agent, was reduced. When, however, carbon tetrachloride and fluroacetate were administered *in vivo*, citrate accumulation was actually higher when both agents were administered to the same animal. The fact that *in vivo* carbon tetrachloride did not produce a loss of citrate from mitochondria was interpreted as a lack of action on the permeability of these particles.

Other investigators have studied the early effects of carbon tetrachloride poisoning. Thielmann *et al.* (156) have reported that two hours after the administration of the hydrocarbon there was an alteration in the response of mice to a glucose tolerance test. This was seen as a decrease in glucose utilization, apparently due to decreased uptake by muscle, and an increase in liver with accumulation of glucose-6-phosphate in this tissue. The authors point out that the same alterations could be achieved by fasting the animals for two hours. This suggests the involvement of humoral factors. Rossi & Zatti (157) have found that 5 hr after 4 g/kg of oral carbon tetrachloride an inhibition of the activation of fatty acids for degradation occurred. Liver fat began to increase significantly after about 3 hr. It is difficult to interpret these results in terms of cause and effect. Börnig *et al.* (158) reported that 2 hr after the poison was administered there was an increase in RNA in the soluble fraction of rat liver homogenate, and an increase in total nucleic acids in one and a decrease in another mitochondrial fraction. It should be emphasized that the hydrocarbon was given intraperitoneally in these experiments. Other early changes in liver metabolism which have been observed with carbon tetrachloride were a decrease in the disappearance of alcohol from rats [Lange (159)], and the release of "quinine oxidase" into the serum of rabbits [Villela (160)].

Late cytochemical changes in the liver certainly involve mitochondrial damage. This is clear from the work cited above and from more recent investigations. Gallagher (161) has reported that the injection of tryptophan, and especially of nicotinic acid, two to three days before carbon tetrachloride, reduced the death rate and prevented the reduction in nucleotides from mitochondria isolated from these animals. Finally, Laudahn (162) claimed that the injection of mitochondria (especially fresh, strongly phosphorylating

ones) reduced the loss of liver enzymes, and metabolites into serum after carbon tetrachloride administration.

Toxins of AMANITA PHALLOIDES. The chemistry and toxicology of the toxins of *Amanita phalloides* have recently been reviewed by Wieland & Wieland (163). Phalloidin and amanitin have been identified as cyclic peptides which are very potent poisons with death resulting from doses in the range of 1 mg/kg. A variety of symptoms were noted including hemorrhagic necrosis of parenchymatous organs, and rapid and complete mobilization of liver glycogen. These symptoms were associated with transient hyperglycemia followed by profound hypoglycemia. While inhibition of a variety of liver enzymes was demonstrated, those of glycolysis were not among them. Thus, the problem of determining the site of action of these poisons is similar to that which has been encountered in the study of carbon tetrachloride. It is necessary to establish the sequence of biochemical alterations which are ininduced by the agents.

Decken *et al.* (164) have found that 2 hr after the intravenous administration of 0.5 to 1 mg/kg of phalloidin to mice and to guinea pigs there occurred a considerable reduction in the rate of incorporation of labeled amino acids into the soluble protein of liver cells. This was also demonstrated by the addition of 10^{-6} *M* phalloidin to microsomes *in vitro*. If ribonucleoprotein containing particles were separated from the microsomes, their capacity to stimulate protein synthesis was not affected by the poison administered *in vivo* (i.e. the site of action of phalloidin appeared to be on some other component of the microsomes). Mitochondrial functions such as oxidative phosphorylation, etc. were not affected 2 hr after administration of the poison. However, they were inhibited at the stage of hepatic hemorrhagic necrosis, several hours later. When liver microsomes were incubated with a high (5×10^{-4} *M*) concentration of phalloidin, inhibition of the incorporation of amino acids into protein occurred only in the presence of added reduced NADP. This suggested that the specificity of the action of the poison on microsomes was due to its conversion by the drug oxidizing system of these particles to an active inhibitor. Matschinsky *et al.* (165) have recently demonstrated that perfusion of the rat liver with phalloidin produced hepatic biochemical and pathological effects similar to those seen *in vivo*. This indicated that the poison acts directly on the liver. Furthermore, a sequence of changes was observed beginning with (*a*) decreased bile formation, (*b*) histological degeneration, (*c*) reduced liver glycogen and ATP levels, and (*d*) increased magnesium activated ATPase activity.

Matschinsky & Wieland (166) have performed some experiments from which they conclude that phalloidin disturbs the structure of liver cells so as to release a series of enzymatic splitting reactions. This results in the loss of essential moieties, and consequently cell death. This hypothesis is based on the finding that 5 hr after administration of the poison to rabbits, soluble and mitochondrial enzymes and fragments of nucleotides, but not the nucleotides themselves, appeared in serum, while there was marked depletion of

liver NAD and ATP. The alterations in the metabolism of mitochondria isolated from the livers were thought to be indirectly related to the action of phalloidin. Obviously, much additional work is required to establish the site of action of the *Amanita phalloides* toxins.

Pyrrolizidine alkaloids.—Several publications have appeared from Australia on the biochemical effects of the pyrrolizidine alkaloids. These substances were isolated from the plant *Heliotropum europaeum*, the ingestion of which by sheep had been known to produce liver damage. The alkaloids have been demonstrated to form complexes with copper [Farrington & Gallagher (167)]. Bull *et al.* (168) have shown that chronic administration to rats of the pyrrolizidine alkaloids, lasiocarpine (8 mg/kg), and heliotrine (27 mg/kg), caused chronic liver damage, while acute fatal necrosis was produced with an LD_{50} of 77 and 269 mg/kg respectively (169). Gallagher (170) then reported that the two alkaloids inhibited rat liver mitochondrial oxidation when present at 3 to 50×10^{-3} M concentration, but were ineffective on succinoxidase and cytochrome oxidase. These results, the finding that the addition of NAD reversed the actions of the alkaloids, and other experiments led to the conclusion that the effect of the poisons was to increase the permeability of mitochondria to the nucleotide. Christie *et al.* (171) demonstrated, furthermore, that the defect in NAD-linked respiration was due to a lack of available nucleotide in the mitochondria, and not due to a failure of NAD synthesis in the whole cell. The evidence for this conclusion was that while pyridine nucleotides fall 50 per cent in 24 hr in heliotrine poisoned animals, a dose of nicotinamide sufficient to maintain normal NAD levels did not prevent the deficiency in mitochondrial respiration. This could only be restored by the addition of NAD *in vitro*.

Antihistamines and neuromuscular blocking agents.—Judah has examined the effects of antihistamines on mitochondrial swelling (172), and on the associated liver damage (173). Promethazine (Phenergan), diphenhydramine (Benadryl), and diphenylpyraline (Hispril) prevented mitochondrial swelling which had been produced by hypotonic sucrose, thyroxin, and calcium when the drugs were added in a concentration of from 5×10^{-5} to 5×10^{-4} M. These drugs also blocked the effect of ATP in reversing such swelling, and the hydrolysis of ATP which occurs simultaneously with the contracting effect. Interference with both mitochondrial swelling and contraction occurred at concentrations of the drugs which did not uncouple oxidative phosphorylation. The effect of these drugs on the liver damage produced by thioacetamide *in vivo* and on the loss of proteins and malic dehydrogenase from slices prepared from such livers was also studied (173). Promethazine prevented the liver damage *in vivo*, and reversed the loss of malic dehydrogenase when added to slices from poisoned livers at a concentration of 10^{-4} M. A reduction of calcium in the incubation medium also prevented the effect of thioacetamide, and in a poisoned perfused rat liver the accumulation of calcium was prevented by promethazine. The mechanism whereby the antihistamine was thought to act was to inhibit mitochondrial

swelling induced by the excess of calcium attributed to the action of thioacetamide. However, the author points out that promethazine was effective in preventing the loss of protein from poisoned slices only if the latter were incubated in a bicarbonate, and not in a phosphate containing Ringer's solution.

Trialkyltins

Trialkyltins are very toxic agents [Barnes & Stoner (174)]. Their study has been continued by Aldridge & Threlfall (175), and Cremer (176) whose work has been confirmed and extended by Moore & Brody (177, 178). These compounds are potent inhibitors of respiration in brain and liver slices after *in vivo* administration or *in vitro* addition to the incubation medium, and also exhibit an uncoupling effect on oxidative-phosphorylation in liver, and brain homogenates, and mitochondria when added *in vitro*. In rat liver mitochondria, ATPase activity is inhibited (177) as is the P_i-ATP exchange reaction (175). Swelling is induced in liver mitochondria which may be prevented by DNP, cyanide or salicylate, partly prevented by ethylenediaminetetracetate, and amobarbital, and reversed by ATP especially in the presence of manganese (178). These effects take place at concentrations which obtain *in vivo* in poisoned animals. Further the mitochondria can accumulate the alkyltins from the medium (175). Aldridge & Threlfall (175) postulate that these agents react with a labile intermediate in the oxidative-phosphorylative sequence which is common to all three steps of the electron transport system at which phosphorylation takes place [see Lehninger (179)], and which may be compared to that postulated for DNP [Borst & Slater (180)]. These effects may also be compared to those of chlorpromazine which are discussed elsewhere in this review; but Cremer (176) has shown that while triethyltin and chlorpromazine both inhibit oxygen uptake, and creatine phosphate synthesis in brain slices, and cause a leakage of creatine, only chlorpromazine produces the effect in diaphragm. The halo- and nitrophenols stimulate oxygen uptake while decreasing creatine phosphate synthesis in these preparations. All of these agents inhibit the response to electrical stimuli in the phrenic nerve-diaphragm preparation, but with a fall in creatine phosphate in all cases except for triethyltin. Thus, it appears that effects on oxidative-phosphorylation may not be the entire explanation for the toxicity of the trialkyltins, and it would be of some interest to determine their effects on excitable membrane phenomena by direct microelectrode measurements. It also appears that in frog sartorius muscle DNP can completely block oxidative-phosphorylation without altering excitability [Abood, Koketsu & Noda (181)].

Antibiotics

The following discussion is based on a selection of articles which have appeared on the mechanism of action of antibiotics.

Strominger and his collaborators have continued their analysis of the site

of action of penicillin with a demonstration of the specificity of the inhibition of incorporation of substrates into the "basal structure" of the bacterial cell wall [Nathenson & Strominger (182)]. Penicillin reduced the incorporation of labeled lysine and inorganic phosphate into the cell wall, but not into the cell protein and nucleic acids of *S. aureus*. In an *E. coli* mutant which required α,ϵ-diaminopimelic acid, the drug inhibited the incorporation of this amino acid, but not that of glucose, valine and phosphate into the cell wall. These results are interpreted as indicating that penicillin has a specific action in interfering with the elaboration of the "basal structure" of the cell wall. This structure consists of acetylglucoseamine, its lactic acid ether and alanine, glutamic acid, and either lysine or diaminopimelic acid. Those substrates whose incorporation into the cell wall was not modified by penicillin make up part of the "special structure." Similarly the antibiotic does not interfere with the synthesis of intracellular protein and nucleic acid. In contrast, gentian violet appears to be a less specific poison, because it does inhibit the incorporation of isotopes into cell protein and nucleic acids.

Two groups have reported on the site of action of cycloserine (D-4-amino-3-isoxazolidine, Oxamycin). Barbieri *et al.* (183) found that the D-isomer inhibited the incorporation of D,L-alanine-1-C^{14} into *E. coli* protein, and especially into cell wall. On the other hand, L-cycloserine, at concentrations at which the D-isomer inhibited, stimulated the incorporation of D,L-alanine. In another paper (184) this group reported that the L-, but not the D-isomer non-competitively inhibited transaminases which acted on L-alanine. This was demonstrated in both *E. coli* and rat liver. The stimulatory effect of L-cycloserine on the incorporation of D,L-alanine into bacterial protein could then be explained on the basis of a reduction of transformation of the L-amino acid to carbohydrate. Strominger *et al.* (185) have determined that the specific action of D-cycloserine is the competitive inhibition of two enzymatic reactions which precede the incorporation of D-alanyl-D-alanine into the "basic structure" [see (182) above] of *S. aureus* cell wall. The first enzyme is alanine racemase, and the second is the dipeptidase which acts on the D-isomer of the amino acid. The result of these inhibitions is the accumulation of the "basic structure" precursor, uridine diphosphate-glucoseamine-lactic acid ether-peptide. The high ratio of K_m to K_i for the dipeptidase reaction is in accord with the effectiveness of D-cycloserine as an antibiotic.

The interaction of chloramphenicol and streptomycin was first observed by Anand & Davis (186), and Anand *et al.* (187) who showed that the former antibiotic blocked the lethal action of the latter, and also prevented the accumulation of labeled streptomycin in *E. coli*. Hurwitz & Rosano (188) have extended these observations by demonstrating that prior addition of chloramphenicol to the culture prevents the lethal action of streptomycin. However, if the cells are first exposed to the latter drug for 1 hr, then subsequent exposure to chloramphenicol and varying amounts of streptomycin results in viability which is inversely proportional to the streptomycin con-

centration. These results are interpreted as showing that during the initial exposure to streptomycin an induction takes place which prepares for the subsequent lethal action of the antibiotic, the induction being reversed or blocked by chloramphenicol.

Slechta (189) has studied the mode of action of the antibiotic psicofuranine in *E. coli*. He concluded that the drug inhibited the conversion of xanthine-5-phosphate to guanylic acid on the basis of the following evidence. The incorporation of glycine-1-C^{14} into guanine was inhibited with a simultaneous increase of the label in xanthine. Similarly the conversion of hypoxanthine and xanthine into guanine and adenine were reduced, the cells excreted xanthosine into the medium, and the inhibition of cell growth by psicofuranine was reversed by guanine but not by other purines or pyrimidines. Some evidence as to the site of action of kanamycin has been collected by Tsukamura (190). One μg/ml of the drug inhibits the incorporation of P^{32} into nucleic acids and proteins and of S^{35} into the trichloracetic acid soluble and insoluble fractions of *Mycobacterium avium*. In a resistant strain the inhibition is not observed. In contrast to kanamycin, neomycin and dihydrostreptomycin inhibited the incorporation of P^{32} into the trichloracetic acid soluble fraction of the cells of the sensitive strain. The antimycotic extract of streptomycete culture, amphotericin B (Fungizone) has been investigated by Gale (191). At a concentration of 5 to 50 μg/ml this antibiotic depressed oxidation and anaerobic glycolysis of intact *Candida albicans*. In a cell free system these effects were lost, suggesting that the drug affects the permeability of the cells.

The action of antibiotics on oxidative phosphorylation has received some attention in the past year. Tappel (192) has reported on the structural requirements for inhibition by antimycin A derivatives of the electron transport chain between succinate or reduced NAD and cytochrome *c*. These were: (*a*) an available ligand group; and, (*b*) a lipophilic group (C_6 to C_9 side chain). This suggested that inhibition was caused by chelation of non heme iron (which has been found in this portion of the electron transport system) in a lipoid environment. Alkyl hydroxy napthoquinones might act in a similar manner. However, inhibition by these compounds of a succinoxidase-coenzyme Q preparation was reversed by the addition of quinones which indicated the possibility of a competition between the inhibitors and coenzyme Q. Welling *et al.* (193) have described the effects of bongkrekic acid (an antibiotic from *Pseudomonas cocovenenans*), on heart muscle homogenate and intact animals. *In vitro* at 10^{-6} *M* concentration it inhibits the oxidation of several substrates such as α-ketoglutarate, while stimulating the oxidation of succinate and β-hydroxybutyrate. At the same time, uncoupling occurs including the phosphorylation which occurs at the level of substrate oxidation of α-ketoglutarate.

Other antimicrobial agents.—Moses *et al.* (194) have reported an interesting effect of α-amino alkylsulfonic acids. These compounds inhibited bacterial growth, but this was quickly followed by spontaneous reversal of the inhibition. It was entirely prevented by the addition of a mixture of amino

acids. This was interpreted as indicating that the α-amino alkylsulfonic acids were antimetabolites of α-amino acids, but were rapidly metabolized to amino acids so that they reversed their own inhibitory effects.

The recently noted high seasonal incidence of cutaneous porphyria in Turkey is probably not a genetically determined disease, but instead is the result of the ingestion of the fungicide hexachlorobenzene (not the insecticide 1,2,3,4,5,6,-hexachlorocyclohexane) according to Schmid (195), and Ockner & Schmid (196). Feeding of the hexachlorobenzene to rats produced a disturbance in porphyrin metabolism characterized by an increase in the amount of porphyrins and their precursors in liver and excreta plus histopathological evidence of hepatocellular degeneration.

Insecticides and Antiparasitic Agents

Insecticides.—Rotenone is a poison which has been used to exterminate both fish and insects. Lindahl & Öberg (197, 198) have produced evidence that this drug inhibits respiration at the level of diaphorase. Exposure of the intact gills of fish after excision to 3×10^{-7} *M* rotenone inhibited their oxygen consumption, and this could be reversed by methylene blue. Rat liver mitochondrial respiration was also inhibited by the poison at concentrations in the range of 10^{-7} to 10^{-6} *M*. Again methylene blue reversed the inhibition of pyruvate oxidation in this preparation. However, when succinate was the substrate, no inhibition by rotenone could be shown. Finally, spectral analysis confirmed that the cytochromes a_3, a, c and b were in the oxidized state in the presence of the drug and partial aerobiosis. The similarity in parts of the structure of rotenone to that of vitamin E and the vitamin K type of quinones may be a clue to the specific site of action of the drug. Winteringham and his collaborators (199) have investigated the effects of DDT, and of dieldrin on the metabolism of the housefly. In the late stage of poisoning which follows hypermotor activity there was a marked reduction in ATP, and a concomitant rise in inorganic phosphate and AMP, while endogenous substrates for energy metabolism were unchanged. Injection of glucose into the prostrate flies sometimes revived the animals and increased the depressed ATP. On the other hand, supression of the convulsive stage by cyclopropane anesthesia neither prevented the fall in the nucleotide, nor the dessication associated with DDT poisoning. The authors believe that dessication is associated with a decreased ability of the fly to utilize endogenous oxidizable substrates with a consequent fall in ATP. In dieldrin poisoning of *Musca domestica*, ATP did not fall after the convulsive stage, but, as with DDT, α-glycerophosphate was markedly reduced. The significance of this finding was discussed in relation to the postulated role of this substance as a carrier of electrons between a soluble α-glycerophosphate dehydrogenase and a structurally bound oxidase.[6]

Although in mammalian liver preparations 2-diethylaminoethyl-2,2-diphenylvalerate (SKF 525A) blocked the activation of both phosphodiamide

[6] An extensive discussion of this possible mode of drug action is given by G. E. Boxer and T. M. Devlin (*Science*, **134**, 1495 (1961).

and phosphorothionate anticholinesterases, *in vivo* parathion poisoning was not blocked. O'Brien (200) has examined this paradox, and has resolved it with the finding that *in vivo* in both mice and cockroaches the SKF compound reduced the inactivation of injected paraoxon, and increased the concentration of this active anticholinesterase after the administration of parathion. The variability of SKF 525A as a protector against or synergist with these organophosphorus compounds is due to the difference in the degree of inhibition of the activation of the inactive precursor or degradation of the active product in the animal.

Antiparasitic drugs.—Bueding *et al.* (201) have examined the biochemical effects of the antihelminthic drug, dithiazine, in the canine whipworm, *Trichuris vulpis*. Under conditions for optimal survival, 0.1 to 0.2 μg/ml of the drug did not inhibit motility of this roundworm, but irreversibly blocked the uptake of glucose by the parasite. Evidence that the action was on glucose uptake rather than utilization was as follows: (*a*) glucose removal by the worm from the medium was reduced, (*b*) the concentration of glucose and other carbohydrates and of ATP in the nematode was reduced, and (*c*) the drug had no effect on the hexokinase of the worm.

Miscellaneous

Protoveratrine and cocaine.—Kini & Quastel (202) have extended their work on the transformation of glucose to amino acids by rat brain cortex slices. They had previously shown that an increase in the K^+/Ca^{++} ratio in the medium stimulates respiration because of an acceleration of the rate limiting step of pyruvate oxidation by NAD to acetyl-CoA and results in increased yields of labeled glutamate, glutamine, and γ-aminobutyric acid from glucose-U-C^{14}, the effect being very sensitive to malonate or amobarbital. They now find that protoveratrine (5 μM) has this same effect which they attribute to the immobilization of, or competition with Ca^{++} resulting in a virtual increase in the K^+/Ca^{++} ratio. Cocaine (0.5 mM) antagonizes the effect of protoveratrine which the authors attribute to a Ca^{++} replacement. However, if higher concentrations (4 mM) of cocaine are used, then even though the respiratory rate falls about 20 per cent, the labeling of amino acids is increased, presumably because more α-ketonic acids are available for transamination. In general the authors feel that the effects of the two drugs may be correlated with their neurophysiologic actions on ion permeability, but differences in the detail of the labeling pattern led them to invoke the dual role for cocaine pointed out above. Recent studies by Chain, *et al.* (203) have pointed up variables which must be taken into account in the interpretation of results obtained with the potassium stimulated system as measured by label distribution from glucose-U-C^{14} in that slices from different areas of the brain show quantitative differences. This should not affect the results of Kini & Quastel, however, since they worked exclusively with cortex slices.

Local anesthetics.—This topic has beeen reviewed by Watson (204) and

papers on the mode of action of this class of drugs which have appeared since then have been mainly concerned with neuronal membrane components. Skou (205), for example, discusses local anesthetic interaction with the lipid components of the membrane leading to a block of the increase of the permeability to sodium ion which accompanies neuronal conduction. Combination of local anesthetics with "acetylcholine receptor protein" has also received attention e.g., (206, to 208).

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