deficient by thiamine-deprivation and by administration of the thiamine antagonists oxythiamine (OTh) and pyrithiamine (PTh). At the onset of symptoms, rats were sacrificed and the oxidative decarboxylation of various α-ketoacids studied in the tissues by a spectrophotometric method with $Fe(CN)_6$ as the electron acceptor. In liver mitochondria thiamine deprivation, OTh-treatment, and PTh-treatment all caused a reduction in the rate of oxidative decarboxylation of pyruvate (to 24·3, 46·2 and 43·1 per cent of the normal rate, respectively). The rate with α-ketoglutarate was affected only by thiamine-deprivation (66·2 per cent of normal). With α-ketoisovalerate and α-keto-β-methylvalerate, none of the deficiencies had a significant effect. In kidney homogenates all three types of deficiency caused a marked reduction in pyruvate oxidation (41·8, 44·3 and 37·9 per cent of normal, respectively) but only thiamine-deprivation and PTh-treatment caused a decrease in α-ketoglutarate oxidation (45·5 and 65·2 per cent, respectively). OTh had no effect. In brain homogenates PTh-treatment caused a decrease in both pyruvate and α-ketoglutarate oxidation (to 48·0 and 50·6 per cent of normal, respectively), while there was no change in thiamine-deprived and OTh-treated brains. The decreased rates of oxidative decarboxylation could be largely restored to normal by the *in vitro* addition of cocarboxylase. Brain and kidney homogenates did not oxidise α-ketoisovalerate and α-keto-β-methylvalerate.

70 Metabolic Pathways of Tetraiodothyro- and Triiodothyro-Acetic and Propionic Acids. E. V. Flock, J. L. Bollman and G. H. C. Stobie (U.S.A.).

Major metabolic pathways for thyroxine (T4) and 3:5:3′ triiodothyronine involve removal of an iodine atom from the benzene ring with the side chain and thus inactivation of these hormones. Conjugates of the products 3:3′:5′ triiodothyronine and 3:3′ diiodothyronine are excreted in the bile of dogs with livers but accumulate in the blood and urine of dogs without livers. Analogues of the thyroid hormones, labelled with ^{131}I in the 3′ or 5′ position, were studied in dogs with biliary fistulas and in dehepatized dogs. In dogs with biliary fistulas tetraiodothyroacetic acid (TETRAC) was metabolized much more slowly than T4; much less ^{131}I was excreted in bile or urine. Large amounts of unchanged TETRAC with smaller amounts of 3:3′:5′ TRIAC were found in the blood. Tetraiodothyropropionic acid (TETPROP) was partially deiodinated to 3:3′:5′ TRIPROP which accumulated in the blood as the amount of unchanged TETPROP decreased. Both of these compounds of propionic acid were excreted in bile as glucosiduronides. Triiodothyroacetic acid and 3:5:3′ triiodothyropropionic acid were rapidly cleared from the blood and excreted in the bile chiefly as glucosiduronides, with small amounts of the sulphoconjugates of 3:3′ diiodothyroacetic acid and 3:3′ diiodothyropropionic acid. In dogs without livers, the sulphoconjugates of these 3:3′ diiodo- compounds were found in larger amounts in both blood and urine, but the major metabolites appeared to be sulphoconjugates of 3′ monoiodo-derivatives.

71 Relations between Structure, Velocity of Biological N-hydroxylation and Toxicity of Aromatic Amines. H. Uehleke (Germany).

N-hydroxylation of aromatic amines in the body forms highly toxic methemoglobin forming hydroxylamine derivatives and seems to be important in allergy and carcinogenesis. Liver microsomes in the presence of TPNH and oxygen can perform the reaction. This is the first time that enzymatic N-hydroxylation has been achieved *in vitro* and that one of the few toxication mechanisms has been elucidated biochemically. The velocity of N-oxydation *in vitro* and *in vivo* depends on the chemical structure of aromatic amines. Aniline, naphthylamine and 2-aminofluorene are slowly oxydized. P-substitution of aniline by electron-attracting groups increases the velocity of N-hydroxylation in the animal and *in vitro*. Compounds with ionizable *p*-substituents (-COOH,-SO_3H) are not measurably attacked, presumably because of fat insolubility. Mono-N-alkylanilines are hydroxylated faster than aniline, the alkyl being removed as aldehyde in the reaction. Levels of oxydized amines in the animal and velocity of N-hydroxylation by liver microsomes are compared with toxicity and methemoglobia forming capacity of the corresponding amines. The reaction of N-hydroxylation will be discussed in view of other known hydroxylation mechanisms of aromatic compounds. The toxication mechanism described gives us a better understanding of the toxicity and side effects of many drugs.

72 Mechanisms of Activation and Induction of Rat Liver Tryptophan Pyrrolase. O. Greengard and P. Feigelson (U.S.A.).

The parenteral administration of a number of agents cause an increased level of tryptophan pyrrolase activity in the liver of rats. These agents include hormones, drugs and the substrate of the enzyme, tryptophan. The elevation of enzyme activity can be as much as 10-fold.

Tryptophan pyrrolase is an unusual heme-enzyme in that in normal liver much of it exists in the free apo-protein form, the presence of which can be revealed by the addition of hematin to the assay mixture.[1] Increased activity can therefore result both from saturation wth cofactor or from increased apo-protein concentration. Methods have been developed to distinguish between the two mechanisms, and differentiation between the modes of action of the various inducing agents has been achieved. The administration of cortisone or reserpine results in the accumulation of the apo-enzyme form of tryptophan pyrrolase in the liver. However, upon

the administration of tryptophan, allyl*iso*propylacetamide and 3-amino-1:2:4-triazole, saturation with the hematin cofactor occurs, *in vivo*, with a subsequent net increase in enzyme protein, in the form of active holoenzyme.

Studies, both *in vitro* and *in vivo*, showed that the association between apo-tryptophan pyrrolase and hematin is facilitated by tryptophan, suggesting that the concentration of the latter may determine what fraction of the existing apo-protein is catalytically active.

1. FEIGELSON and GREENGARD (1961), *J. Biol. Chem.*, **236,** 153.

73 Extraction of a Seizure Substance (K Substance) from the Dog's Brain. T. HAYASHI and K. NAGAI (Japan).

When an electro-shock current was applied through the skull of dogs, a generalized seizure appeared, which continued 60–180 sec after the cessation of the stimulation. During these seizure, a seizure substance (K substance) was released from the motor cells which rapidly diffused into the cerebrospinal fluid. Fluid thus obtained was introduced into the c.s.f. of another dog; it produced a clonic convulsion in the receiver dog. There were, however, a few difficulties with these experiments, as success did not occur in every case. Recently, Hayashi *et al.* succeeded in all cases, if the fluid was treated with absolute methyl alcohol after reduction of its volume by a third. After centrifugation the fluid was reduced by evaporation in a 40°C water bath to 30 per cent of the original volume. This fluid contained K substance, and when it was introduced into the ventricle of the receiver dog a strong seizure was produced. The K substance could be extracted from the grey matter of the cortex, but not from the white matter.

The active agent was highly unstable in an oxygen atmosphere, but when it was kept in contact with nitrogen the activity was maintained for a long time. Purification and chemical analysis of it is now being carried out in our laboratory.

74 The Physiological Action of K Substance Extracted from Dog's Brain. T. HAYASHI and K. NAGAI (Japan).

The active agent[1] which was contained in the cerebrospinal fluid of dogs taken during seizure induced by electric stimulation was examined.

When a small amount of it was injected into the exposed motor cortex of a dog, a generalized seizure was produced, as when an electric stimulation was applied. For instance: (a) when concentrated, the clonic convulsion; (b) when diluted, the locomotive convulsion (walking movement); and (c) when more diluted, a tonic movement or twitch of one limb.

When it was introduced into a ventricle, it produced seizure with latent period of 3–20 sec in the receiver dog. When it was rapidly injected through the carotid artery, a seizure appeared with latent period of 2–5 sec.

The agent had no action on the respiration and blood pressure of dogs when injected intravenously. It had no action on an excised toad's intestine, nor on an excised heart preparation.

The action of K substance was neutralized by mixing of an appropriate dose of γ-amino β-hydroxybutyric acid, which we believe was the real inhibitory transmitter of the central nervous system of higher animals. Thus we presume that the agent is the "excitatory transmitter" in the central nervous system of mammals.

1. HAYASHI *et al.* "Extraction of a seizure substance (K substance) from dog's brain" in this congress.

75 γ-Aminobutyric Acid and Vagal Respiratory Regulation. J. A. SCHNEIDER and A. B. DRAKONTIDES (U.S.A.).

γ-aminobutyric acid (GABA), a normal brain constituent, was reported several years ago to have an inhibitory influence on crustacean stretch receptors.[1] Only recently, however, inhibitory or "desensitizing" effects of GABA on slowly adapting pulmonary stretch receptors of the cat were described.[2] Since these receptors are thought to be part of the ascending arc of the Hering–Breuer Reflex, a study of the action of GABA on vagal respiratory reflexes was indicated. Simultaneous recording of whole body plethysmography and discharges from afferent pulmonary stretch receptors in cats were used to analyze respiratory characteristics during GABA injection. Tracheal occlusion responses were utilized to assess vagal respiratory function.

The study indicates that an increase in depth of the inspiratory phase and a transient elevation of the absolute lung volume coincides with the desensitizing effect of GABA on pulmonary stretch receptors. Tracheal occlusion responses, however, were not altered consistently. The relatively short duration of respiratory changes following intravenous GABA administration contrasts markedly with the prolonged inhibitory effect on pulmonary stretch receptors, indicating rapid adjustment of vagal respiratory regulation. The possible physiological significance of GABA in the regulation of respiration remains unclear.

1. (1954), *Arch. Int. Physiol.*, **62,** 33.
2. (1960), *Amer. J. Physiol.*, **199,** 748.