tion in 11 out of 20 experiments. When present, this effect is dose-dependent and it could be antagonized by nicotinic acid (Figure). In contrast to this, CAMP in concentrations from 10^{-5} M to 2×10^{-3} M did not affect the response of the diaphragm to indirect stimulation in all 8 experiments. These findings are in agreement with the action of these 2 nucleotides on the concentration of glycogen in diaphragm (Table). CAMP itself did not change the concentration of glycogen in diaphragm, whereas db-CAMP produced a significant decrease.

It should be pointed out that even CAMP produced potentiation of the diaphragm contractions if added to the bath after previous addition of adrenaline (2×10^{-7} g/ml). Similarly, a potentiating effect of CAMP and adrenaline in producing glycogenolysis in diaphragm was also observed (Table).

These experiments indicate that CAMP might act as a 'second messenger' mediator in producing facilitatory responses of the isolated diaphragm to indirect stimulation. The activated metabolic processes might have the predominant role in this response.

Résumé. Le dérivé dibutyrique du 3′, 5′-AMP cyclique potentialise les contractions du diaphragme provoquées par stimulation électrique indirecte, alors que l'application du 3′, 5′-AMP cyclique n'a pas un tel effet. D'autre part, ce dérivé diminue le taux de glycogène du diaphragme, ce qui n'est pas le cas pour le 3′, 5′-AMP cyclique.

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Albumin Content of Hepatocytes in Experimental Cirrhosis

Serum albumin concentration is often reduced in cirrhosis, and this has been attributed to depression of albumin synthesis following hepatocellular damage[1]. We have employed the fluorescent antibody technique in an attempt to determine whether the production of experimental cirrhosis is accompanied by a reduction in the intracellular albumin content of the liver.

Cirrhosis was induced in 4 male Charles River rats weighing approximately 290 g using the method of MCLEAN et al.[2], sodium phenobarbitone being given for 1 week prior to twice weekly exposure to carbon tetrachloride. This combined treatment was continued for a period of 8 weeks. Since dietary protein intake is known to influence the intracellular albumin content of the liver[3], 4 untreated control rats of the same species, sex and weight were pair fed with the test animals during the entire experimental period of 10 weeks. At the end of the 10th week i.e. 1 week after cessation of treatment both groups of rats were fasted for 2 h and killed under ether anaesthesia. Blocks of liver were immediately taken, fixed in ice-cold 95% ethanol containing 1% acetic acid and processed after the method of HAMASHIMA et al.[4].

Rat albumin was prepared by ammonium sulphate precipitation and then purified by ion-exchange chro-

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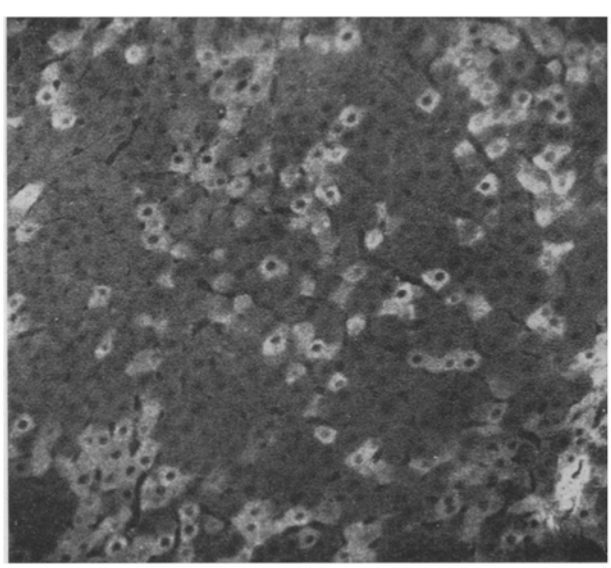

Fig. 1. Normal rat liver showing numerous randomly distributed fluorescent cells. ×163.

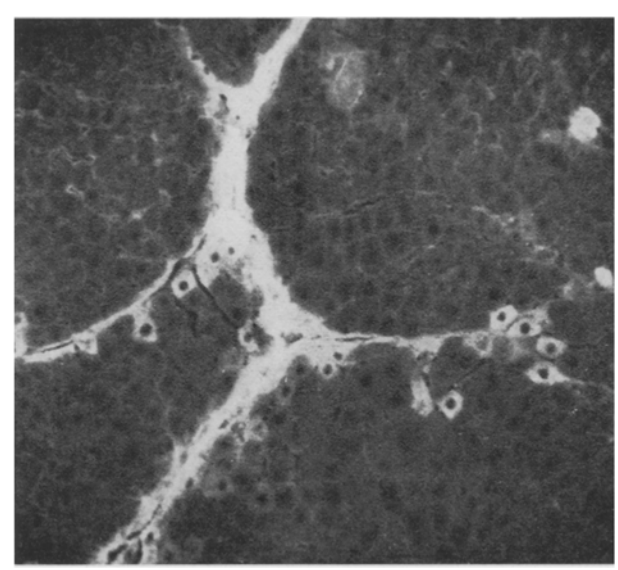

Fig. 2. Cirrhotic rat liver showing only a few fluorescent cells mainly around the periphery of the nodules. Note also the bright staining of the fibrous tissue septa. ×163.

matography (DEAE Sephadex A-50 with NaCl gradient elution). This latter step was repeated twice and the resulting product was shown to be homogeneous by immunoelectrophoresis. Rabbits were immunized with this antigen emulsified in Freund's adjuvant. A globulin precipitate was prepared from the resultant antiserum[5] and this was then conjugated with fluorescein isothiocyanate. Unconjugated material was removed by passage through a G25 Sephadex bed and staining specificity improved by DEAE cellulose column chromatography[6].

Figure 1 shows the random distribution of fluorescent hepatocytes typical of the normal rat liver. In each cirrhotic liver e.g. Figure 2 these cells are arranged mainly around the periphery of nodules and are notably reduced in number. In addition there is bright fluorescent staining of the fibrous tissue septa. Absorption of the conjugate with a precipitate of pure collagen obtained from tail tendon[7] failed to abolish or diminish the fluorescent staining of the septa, but staining of both hepatic parenchymal cells and fibrous tissue was completely abolished following absorption with a purified preparation of rat albumin. Serum proteins including albumin are closely bound to connective tissue[8] and this probably explains the non-specific staining of hepatic collagen.

The number of fluorescent hepatocytes in each liver section was estimated by 2 independent observers using a point counting technique. The results show a highly significant fall ($P<0.0005$) in the numbers of fluorescent cells in the cirrhotic livers (Table).

It is uncertain whether the reduction in intracellular albumin reflects decreased storage or synthesis, and also whether this change is attributable to cirrhosis as such, or merely to recent exposure to carbon tetrachloride and sodium phenobarbitone. Further experiments are in progress to clarify these points[9].

Frequency of fluorescent hepatocytes per 1000 in 4 normal and 4 cirrhotic livers

Normal Liver	Observer 1	Observer 2	Cirrhotic Liver	Observer 1	Observer 2
1	133	87	1	50	31
2	116	126	2	67	45
3	120	177	3	98	37
4	103	116	4	35	57
Mean (± SD)	122 ± 26		53 ± 24		

$t = 5.7657$ $P < 0.0005$

Résumé. L'albumine intracellulaire a été démontrée dans le foie de rats par une technique d'immunofluorescence. Il y a une réduction significative du nombre des cellules fluorescentes dans les foies des animaux souffrant de cirrhose produite par le CCl_4.

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Phenethylamine Content of Human Urine and Rat Brain, its Alterations in Pathological Conditions and After Drug Administration

Phenethylamine has been postulated as a physiological stimulating (ergotropic) agent in brain[1,2]. It is a typical substrate of monoaminooxidase, a circumstance that may explain the action of monoaminooxidase inhibitors in depressed patients[3]. The urinary elimination of phenethylamine, studied with paper chromatography, was diminished in endogenous depressions and it was normal or elevated in other mental diseases (schizophrenia, alcoholism)[4].

We carried out assays of phenethylamine in urine with a new quantitative method based on spectrophotofluorometry and controlled by thin layer chromatography[5,6]. The same method was used to study the phenethylamine content of the rat brain. The brains were extracted by a 20% sodium sulfate solution in 0,5*N* HCl. Table I shows the results of assays carried out with endogenous and secondary or atypical (organic, schizoaffective, alcoholic etc.) depressions, before and after treatment with tricylicdibenzepinic antidepressive drugs (chlorimipramine, Merck MK) and of other mental patients.

Table I shows that phenethylamine excretion is significantly diminished in endogenous depressions. In secondary and atypical depressive states, as well as in arteriosclerotic dementia, normal values have been obtained. Abnormally high amounts were found in mania and schizophrenia. During the treatment of endogenous depression with tricyclic-dibenzepinic drugs a normalization of phenethylamine elimination took place, whereas in secondary or atypical depressions no significant changes

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