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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Table I. Phenethylamine content of human urine (μg/24 h)

Diagnosis	No. of cases	Condition	Limits	Main value	SD	S.E.M.
Normal	17	–	105–775	336	180	45
Endogenous depression	14	–	30–225	114	49	13
Endogenous depression treated with tricylic antidepressive drugs	4	Before treatment	90–275	151	24	12
		After treatment	330–555	421	58	29
Secondary and atypical depressions	6	Before treatment	290–1079	643	129	53
		After treatment	320–840	552	78	31
Circular manic-depressive psychosis	1	Depressive phase	275	–	–	–
		Manic phase	2175	–	–	–
Mania	4	–	690–1275	1047	240	180
Schizophrenia	4	–	960–2700	1485	700	410
Temporal epilepsia	3	–	345–1035	620	–	–
Arteriosclerotic dementia	3	–	210–500	346	–	–

Table II. Phenethylamine content of rat brains in μg/g

	Untreated	Iproniazide	Reserpin	Imipramine	Chlorimipramine
No. of animals	10	5	10	5	5
Main value	0.492	1.06	0.116	1.84	1.08
S.D.	0.036	0.246	0.015	0.58	0.32
S.E.M.	0.012	0.130	0.005	0.27	0.15

Differences with the normal value are significant at $p < 0.01$.

were produced. In a circular case of manic-depressive psychosis, a sudden elevation was observed as the state changed from depressive to manic phase. Technical and clinical details will be published elsewhere[5,6].

The results of assays on phenethylamine content of rat brains without and after treatment of the animals intraperitoneally with iproniazide (100 mg/kg, 24 h before), reserpin (10 mg/kg, 18 h before), imipramine (50 mg/kg, 24 h before) and chlorimipramine (50 mg/kg, 24 h before), respectively, are shown in Table II. In each group the brains of 5 or 10 animals were assayed individually.

Table II shows that phenethylamine content of rat brain is significantly elevated by iproniazide, imipramine and chlorimipramine and reduced by reserpin. Similar results were obtained with another quantitative method as to reserpin and imipramine effects[7] and by an independent investigation carried out by other workers elsewhere as to the effects of reserpin and a monoaminooxidase inhibitor[8]. It is not known by what mechanism tricyclic-dibenzepinic drugs elevate phenethylamine concentrations, for they have no monoaminooxidase inhibiting action. Possibly they stimulate the production of phenethylamine. This possibility is being investigated.

Zusammenfassung. Nachweis, dass die Phenethylaminausscheidung bei endogener, nicht aber bei sekundär atypischer Depression signifikativ vermindert, bei Manie und Schizophrenie aber entsprechend erhöht ist. Die Behandlung der endogenen, nicht aber der sekundären Depression mit trizyklischen antidepressiven Mitteln erhöht die Phenethylaminausscheidung. Der Phenethylamingehalt im Rattengehirn wird durch Reserpin signifikant vermindert, durch Iproniazid, Imipramin und Chlorimipramin jedoch signifikant erhöht.

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