cerned, but it does not refute the experimental approach to the main problem (7).

F. B. BENJAMIN
Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia

References and Notes

1. H. K. Beecher, *Pharmacol. Revs.* 9, 59 (1957).
2. J. D. Hardy, I. Jacobs, M. D. Meixner, *J. Appl. Physiol.* 5, 725 (1953).
3. F. B. Benjamin and O. Bailey, Unpublished data.
4. J. D. Hardy, H. G. Wolff, H. Goodell, *Pain Sensations and Reactions* (Williams and Wilkins, Baltimore, Md., 1952).
5. V. Guillemin, F. Benjamin, T. Cornblect, M. I. Grossman, *J. Appl. Physiol.* 4, 920 (1952).
6. T. Lewis, G. W. Pickering, P. Rothschild, *Heart* 15, 359 (1929.)
7. This investigation was supported by U.S. Navy Grant N-ONR-551(12).

17 March 1958

New Chromosome Number for the Order Caudata

During recent investigations of the chromosomes of the ambystomid salamanders occurring in the Pacific Northwest, it was found that one of the members of the family, *Rhyacotriton olympicus,* has a haploid chromosome number of 13; somatic tissues revealed a diploid number of 26. As far as can be determined from the literature (*1*), this constitutes a new chromosome number for the family Ambystomidae, and it is also the first such number recorded for the entire order Caudata. All other ambystomid salamanders known cytologically have $n = 14$, $2n = 28$, as is summarized in Table 1.

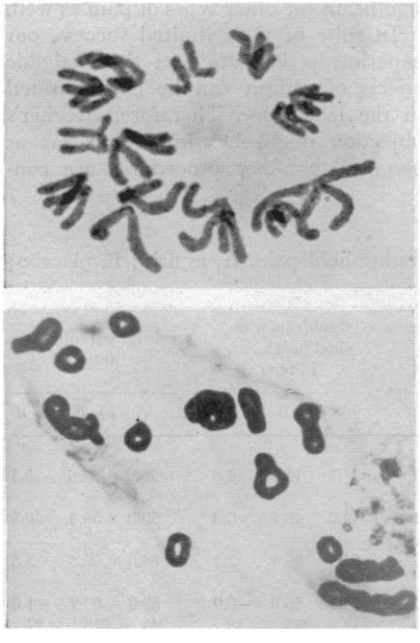

Fig. 1. Chromosomes of *Rhyacotriton olympicus* at meiotic anaphase I (top), showing 13 sets of sister chromatids, and at late diplotene of the meiotic prophase I (bottom), revealing 13 bivalents.

The chromosome numbers for *Rhyacotriton olympicus,* $n = 13$, $2n = 26$, were determined from counts in cells obtained from meiotic testes and from regenerating liver tissue. The material was prepared by a modification of the aceto-orcein squash technique of La Cour. *Rhyacotriton olympicus,* the only species in this genus, is found along the banks of cold streams of the Coast Mountains from Northern California to the Olympic Peninsula (*2*). Counts were made on the cells of 12 individuals taken mainly in the zone of intergradation of the two subspecies *olympicus* and *variegatus,* and at least 20 counts were made per individual.

The morphology of the haploid set reveals eight metacentric chromosomes and five submetacentric ones. If the chromosomes are arbitrarily divided into longer and shorter ones, the set can be formalized for this species as $4M$, $2S$, $4m$, $3s$, where M = metacentric, S = submetacentric; the lower-case letters denote the shorter chromosomes of the set. The longest chromosome at anaphase II averages 19 μ, the shortest averages 6.5 μ. The ratio of the longest chromosome to the shortest for the haploid set is 2.9. Chiasma frequency was determined at diplotene of prophase I to have a mean of 39, with a range of variation from 36 to 42. The number of bivalents showing a minimum of two chiasmata was five. Figure 1 (top) shows the haploid set at anaphase I, and (bottom) the 13 bivalents at diplotene. As in other studies of the Caudata, no evidence for heterochromosomes was found in this species.

In addition to being of interest as a new chromosome number for the order Caudata, this finding allows for some speculation regarding the systematic position of the genus *Rhyacotriton.* While in some groups the chromosome number varies even among species of the same genus, this has not been true of the salamanders. If, as was stated by Matthey (*3*), "A chromosomal discontinuity corresponds to the familial discontinuity of the systematicians; within the families the fundamental homologs of the chromosomes are respected . . . ," it would seem that a taxonomic revision might be indicated. Perhaps this species belongs to a new and separate family. On the other hand, it may be that this merely represents an evolutionary offshoot not divergent enough to enjoy a separate family status but still indicating a genus rather remote from the main group of ambystomids. Other cytological and morphological evidence confirms the rather unique character of this salamander with respect to other ambystomids. In a similar situation in another family of Caudata, the Salamandridae, Fankhauser (*4*) found that American species of *Triturus* have a diploid chromosome number of 22, while European species and the Japanese *Triturus pyrrhogaster* have 24. All these species have been allowed to remain in the same genus, aside from the recent shift of the Pacific Coast species to the genus *Taricha* which was based on priority and not on cytological considerations (*5*).

Table 1. Chromosome numbers in ambystomid salamanders.

Species	Haploid No.	Diploid No.	Investigator
	Ambystoma		
A. mexicanum	—	28	Wickbom, others (*1*)
A. tigrinum	14	28	Parmenter; Carrick (*1*)
A. maculatum	—	28	Henley and Costello (*6*)
A. jeffersonianum	14	—	Kezer (7)
A. gracile	14	28	Humphrey (7)
A. macrodactylum	14	28	Humphrey (7)
	Dicamptodon		
D. ensatus	14	28	Humphrey (7)
	Rhyacotriton		
R. olympicus	13	26	Humphrey

D. G. HUMPHREY
Department of General Science, Oregon State College, Corvallis

References and Notes

1. S. Makino, *Chromosome Numbers in Animals* (Iowa State College Press, Ames, 1951).
2. R. Stebbins, *The Amphibians of Western North America* (McGraw-Hill, New York, 1954).
3. R. Matthey, "The Chromosomes of the Vertebrates," *Advances in Genet.* 4, 159 (1951).
4. G. Fankhauser, *Anat. Record* 54, 73 (1932).
5. This work was supported in part by grants from the AAAS, the American Academy of Arts and Sciences, and the Graduate Council of Oregon State College.
6. C. Henley and D. Costello, *J. Morphol.* 89, 91 (1951).
7. Unpublished data.

10 March 1958

Serum Diphosphopyridine Nucleotide Linked Enzymes in Delirium Tremens and Allied Conditions

During the past few years some transaminase and dehydrogenase enzymes as well as fructoaldolase (aldolase) in biological fluids have received considerable attention in various pathological conditions in man—for example, in myocardial infarction and in acute liver cell damage. These enzymes are widely distributed in the cells of the body, and the working hypothesis is that they are liberated into extracellular fluid in pathological conditions with localized or diffuse cell damage (necrosis) in certain tissues. The amounts of enzymes liberated are then approximately proportional to the magnitude of the tissue damage. The enzymes may be deter-

mined by measuring the change of absorbance at 340 mμ with time for the transformation $DPN^+ \rightleftharpoons DPNH$ when DPN is part of the system or when it is coupled to such a system (*1–3*).

Liver damage is a common finding in alcohol addicts; either a lipomatosis or a cirrhosis of the liver is often found. The immediate cause of delirium tremens (DT) is unknown at present, but some similarities with liver precoma are obvious. To study whether there might be acute liver cell damage in delirium tremens, we have followed about 400 cases of DT and allied conditions with determinations of glutamic-oxalacetic transaminase (GOT) and also, in about 100 cases, of glutamic-pyruvic transaminase (GPT), malic dehydrogenase (MD), and aldolase in serum, during the acute stage of the disorder and in the reconvalescents. The patients have usually shown signs of a chronic liver disease, probably a lipomatosis, as measured by bromosulfophthalein clearance, prothrombin time before and after administration of vitamin K, serum bilirubin, bile pigments in urine, total lipids in plasma and others. Only in about 1 percent of the cases have clinical signs of cirrhosis been obtained.

Markedly elevated serum GOT levels are found in delirium tremens (*4*). The elevation is simultaneous with the mental and somatic symptoms, and a GOT peak is obtained at the height of these symptoms. When the delirious state is over, the GOT level usually reverts rapidly to low or normal values. The peak level is correlated with the severity of the delirium. Usually 200 to 1000 Karmen-Ordell units (*3, 5*) are found in severe cases and less than 300 units in mild and moderately severe cases, in syndrome B, and in syndrome C (see Table 1). In one case, a moderately severe delirium tremens and commotio cerebri without electroencephalographic changes, a peak level of 2900 units was obtained. When patients are admitted during acute alcohol intoxication, before a delirium has started, high GOT levels are also observed. The level then often reverts rapidly to low or normal values as the blood alcohol concentration decreases, but rises again if delirium tremens follows. In alcohol addicts a GOT rise is also often seen after a single day's drinking, even if a long period of abstinence with normal GOT level has preceded the incidence. Two periods of delirium were obtained for a few patients, and then two GOT peaks were found.

Serum GPT and aldolase are also elevated in delirium tremens and allied conditions. Glutamic-pyruvic transaminase is not raised as markedly as GOT. In general, a peak is obtained during the acute stage, but the ratio GPT:GOT is always below 1.0, which is contrary to the case with infectious hepatitis (*6*). Usually GPT does not exceed 100 units, except in severe delirium tremens; the highest level observed in severe cases was 200 units. Now and then zero level was obtained (no pyridoxal phosphate was added during analysis, but the patients were given vitamin B_6). To control this finding, pyridoxal phosphate was added on analysis of serum from other delirium tremens patients, but without any significant effect on the GPT or GOT values. The aldolase level follows approximately the same course as the GOT level, but is more variable from time to time. Maximum values up to 80 Bruns units (*2*) are obtained. Malic dehydrogenase is also elevated during the acute stage, but is variable from time to time (mainly determined indirectly as GOT in serum without added MD, in percentage of GOT value with added excess MD) (*5*). In general, values below 80 percent are obtained, as in disorders other than myocardial infarction. In a few cases lactic dehydrogenase was also determined, but this enzyme was only slightly elevated in serum.

These findings suggest acute liver cell damage in delirium tremens in addition to the chronic liver disease also observed. However, cell damage in other tissues such as skeletal muscle, heart, kidney, or brain cannot be ruled out as an additional cause of the raised enzyme levels in serum. In a few cases GOT was determined in cerebrospinal fluid. The values found were within normal limits (*7*).

Table 1. Maximum values of serum GOT, GPT, and aldolase during delirium tremens and allied conditions in alcohol addicts. The terminology with respect to the acute (mental and physical) sequelae to alcohol abuse in alcohol addicts is confusing in the pertinent literature. We have classified these sequelae as follows (only the most important symptoms are given here) (*4*): Syndrome B: tremor, anxiety, perspiring, bad sleep or bad appetite, or both; no hallucinations or disorientation. Syndrome C: the same, but with hallucinations; no obvious disorientation. Delirium tremens (DT): tremor and other motorical symptoms, marked vegetative disturbances; hallucinations and disorientation; DT_1, mild intensity; DT_2, moderate intensity; DT_3, severe intensity. Alcoholic delirium in geriatric cases (delirium tremens sine tremore): mild DT without tremor. In this table, Mild DT corresponds to $DT_1 + DT_2$ + geriatric alcoholic delirium; Severe DT corresponds to DT_3.

Clinical group	No. of cases	Range of values	Mean and S.E.* of mean	S.D.†	Student's *t* test of significance‡	
					t	*p*
			Glutamic-oxalacetic transaminase			
Normal	25	11– 35	**21.6** ± 1.3	6.1		
Syndrome B	249	18– 348	**81.6** ± 3.7	58.0	5.17	< 0.001§
Syndrome C	39	37– 500	**115.0** ± 16.2	100.8	2.97	< 0.01§
Mild DT	151	16–2920	**183.7** ± 20.0	245.6	1.71	< 0.1
Severe DT	22	228–1020	**494.1** ± 56.0	262.6	5.87	< 0.001§
			Glutamic-pyruvic transaminase			
Normal	24	5– 29	**14.8** ± 1.2	5.8		
Syndrome B	67	4– 166	**51.8** ± 5.3	43.0	4.18	< 0.001§
Syndrome C	11	23– 133	**57.0** ± 12.1	40.1	0.12	> 0.9
Mild DT	52	19– 206	**62.2** ± 5.0	35.9	0.43	< 0.7
Severe DT	7	100– 201	**144.0** ± 15.1	39.9	5.59	< 0.001§
			Aldolase			
Normal	24	5– 13	**8.3** ± 0.4	1.8		
Syndrome B	67	3– 50	**15.5** ± 0.8	6.9	5.05	< 0.001§
Syndrome C	11	6– 48	**19.2** ± 3.6	11.9	1.47	< 0.2
Mild DT	52	6– 71	**26.7** ± 2.0	14.7	1.59	< 0.2
Severe DT	7	19– 60	**36.0** ± 5.9	15.5	1.56	< 0.2

* S.E., standard error. † S.D., standard deviation.
‡ Comparison between pairs of clinical groups: normal versus syndrome B; syndrome B versus syndrome C; syndrome C versus mild DT; mild DT versus severe DT.
§ The difference of means is statistically significant.

LARS-GÖRAN ALLGÉN,
SANDER IZIKOWITZ, BRITTA NAUCKHOFF,
INGA-BRITT ORDELL, INNA SALUM
Beckomberga Mental Hospital,
Bromma, Sweden

References and Notes

1. J. S. LaDue and F. Wróblewski, *Ann. Internal Med.* 43, 345 (1955); A. Karmen, F. Wróblewski, J. S. LaDue, *Science* 120, 497 (1954); F. Wróblewski and J. S. LaDue, *Proc. Soc. Exptl. Biol. Med.* 91, 569 (1956).
2. F. Bruns, *Biochem. Z.* 325, 156 (1954); ——— and W. Jacob, *Klin. Wochschr.* 32, 1041 (1954).
3. A. Karmen, *J. Clin. Invest.* 34, 131 (1955).
4. L.-G. Allgén *et al.*, *Nord. Med.* 58, 1921 (1957).
5. R. Ordell, report presented at the 2nd European Congress of Cardiology, Stockholm, 1956; and personal communication.
6. F. De Ritis, M. Coltorti, G. Giusti, *Minerva Med.* 46, 1207 (1955).
7. This investigation has been aided by a grant from AB Leo, Sweden.

12 May 1958