Effect of Aspirin on Suprathreshold Pain in Man

Beecher, in his extensive review on the measurement of pain (*1*), came to the conclusion that experimental pain in man, as used at present, is not suitable for appraisal of analgesic agents. For investigators interested in the physiology of pain, the evaluation of analgesic agents is a side issue. However, something must be wrong with our concept of pain if the investigator thinks that he can use experimental pain for evaluation of analgesic agents even though practical experience shows that it is not suitable for that purpose. Experimental pain may be different from clinical pain; still it might provide useful information if we realize that it is different and know why.

Beecher (*1*) also pointed out that experimental pain in animals can be used for evaluation of *potent* analgesic drugs. If an injurious stimulus is applied to an animal, a skin twitch, a tail flick, or a withdrawal reaction may be observed. These are spinal reflexes and therefore are side effects of pain. They are even undesirable side effects, for an ideal analgesic agent should eliminate pain without affecting reflex activity. Potent analgesics can be tested by this type of procedure because they are not specific for pain. This explains the failure of weak analgesic agents in tests of animals. It does not explain the failure of experiments with human beings.

In a recent investigation in which Hardy's thermal radiation method (*2*) and double-blind technique were used in man, no difference was found between the effect of 10 grains of aspirin on the pain threshold and that of a placebo (*3*). Pain threshold is fairly stable from individual to individual and is independent of age, sex, race, and emotion (*4*). However, all available evidence indicates that the suffering produced by an equally injurious stimulus varies markedly (*1*). This shows that the determination of pain threshold involves only one aspect of the total pain complex, which might be called pain perception. If the effect of aspirin were on the mechanism of pain perception, we would expect a rise of pain threshold. If aspirin affected the reaction component of pain (*1*) we might not see any effect on the pain threshold, while at greater pain intensities the effect might come out.

This idea was tested in a series of 32 experiments in which four types of pain and double-blind techniques were used. The 16 subjects were males 21 to 44 years of age. Pain was produced by the following procedures:

1) The hand was immersed in ice water. This produces marked pain which increases to a peak within about 20 seconds. After that there are irregular fluctuations until the pain intensity decreases after about 60 seconds. For evaluation the time of occurrence of this peak was used as well as the pulse rate 60 to 75 seconds after immersion (control rate, 79.6 per minute).

2) Hardy's method of radiating the India ink painted volar side of the forearm with thermal radiation (200 mcal/sec/cm^2) was used (*2*). The final skin temperature was calculated from the initial skin temperature, the known radiation intensity, and the measured time of exposure (*2*). Each subject was asked to determine his own intensity, which he considered as marked pain. Any further attempt to fix the stimulus intensity was avoided to allow free reign for the emotional aspect of pain. Two determinations were made for each test. The mean was taken as the test value for the experiment.

3) Contact heat was applied to the skin of the forearm with an instrument that allows heating of a small probe with simultaneous recording of the probe temperature (*5*). The probe was applied to the forearm. The heat was turned on, and the temperature was recorded when the subject reported that he had reached the pain intensity determined by procedure 2. Five determinations were made each time. The two extremes were eliminated, and the mean of the remaining three was considered as the test value.

4) A blood pressure cuff was applied to the upper arm and inflated to a pressure of 200 mm-Hg (*6*). Then the subject started pressing a Veeder counter rhythmically. The subject started with a fast rate but gradually slowed down, until finally ischemic pain made it impossible for him to go on. The number of clicks and the time were recorded. By dividing the number of clicks by the time, a ratio was obtained, the constancy of which appears to be a good indication of the subject's ability to reproduce the same pain intensity. This means that an analgesic agent or at least aspirin will prolong the time and increase the number of clicks to the same extent, so that the ratio remains constant. Out of our 16 subjects four were experienced in this type of work and showed a very steady ratio. Of the other 12 subjects, five showed less than 5 percent change. These differences in evaluation were brought out by considering first the whole group of 16 subjects, then the nine subjects with good pain evaluation, and last the four trained subjects with the best ratios.

During the whole series care was taken not to mention any values or discuss any part of the experiment. In analysis each subject was considered his own control, and the percentage change after administration of aspirin was used as basis for the statistical evaluation.

The results are given in Table 1. Because of the inherent variability of the method, significant changes occur only with the ischemic contraction method of pain production. The number of contractions is increased and the time of continuing rhythmic contractions is prolonged, while the number contraction/time ratio remains the same. The difference between aspirin and placebo becomes more pronounced with the greater ability of the subject to evaluate pain objectively. As the tests using ice water and radiated heat show also an increased mean difference between the aspirin and the control group with better judgment on the part of the subject, this might be an indication that with an increased number of trained subjects the effect of a weak analgesic agent might become significant for other types of pain as well.

In spite of their limited success, our experiments indicate that the analgesic effects of aspirin can be demonstrated in the laboratory. Therefore, Beecher's objection might be justified as far as certain laboratory procedures are con-

Table 1. Effect of aspirin on four types of suprathreshold pain. A, aspirin; B, placebo; *d*, difference.

Pain	16 subjects, 32 tests			9 subjects with good judgment, 18 tests			4 trained subjects, 8 tests		
	A	B	*d*	A	B	*d*	A	B	*d*
Time of peak pain on immersion of hand in ice water (sec)	22.4	20.4	2.0	24.7	19.9	4.8	30.0	24.6	5.4
Radiation heat, final skin temperature (°C).	49.3	49.1	0.2	49.0	48.7	0.3	50.9	50.4	0.5
Pulse rate 1 min after immersion in ice water	84.3	85.9	1.6	84.3	86.4	2.1	84.0	80.2	3.8
Contact heat, final skin temperature (°C)	55.2	55.6	−0.4	55.1	56.0	−0.9	59.9	60.9	−1.0
Number of ischemic contractions	256	250	6.0	252	233	19	245	218	27
Significance (*t* test)	0.02			0.01			0.05		
Time of maintaining ischemic contractions (sec)	84.3	81.9	2.4	80.6	74.4	6.2	77.7	69.2	8.5
Significance (*t* test)	n.s.*			0.02			0.05		

* Not significant.

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.

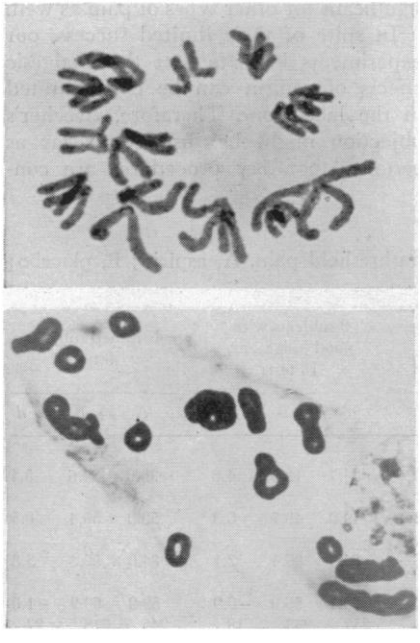

Fig. 1. Chromosomes of *Rhyacotriton olympicus* at meiotic anaphase I (top), showing 13 sets of sister chromatids, and at late diplotene of the meiotic prophase I (bottom), revealing 13 bivalents.

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-