CHANGES OF THE SPONTANEOUS ACTIVITIES OF RATS BY THE ADMINISTRATION OF MORPHINE

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Many studies have been done concerning the influences of morphine on the spontaneous activities of animals. Now it was fairly disclosed that the changes of the spontaneous activity (Sp. Act.) are closely related to the species of the animals, the size of the dose, the interval and the regularity of administration and so on. (Eddy⁽¹⁾, 1941; Schmidt and Livingston⁽²⁾, 1933; Reynolds and Randal⁽³⁾, 1957; Schauman⁽⁴⁾, 1957; Seevers⁽⁵⁾, 1954)

However, the detailed studies on the relationships between the Sp. Act. of rats and the morphine-administration seem to be still lacking.

Few years ago, Yanagita⁽⁶⁾ assembled ingeniously an "electronic activity level counter for small animals" by which the amounts of the Sp. Act. of 4 rats are demonstrated simultaneously, separately and sensitively *by number* as the mileage meter of car or recorded on kymograph-paper as the corresponding length of vertical line as long as 40 hours continuously.

This paper deals with the changes of the Sp. Act. of rats before and after the morphine administration quantitatively using this instrument, with special emphasis on its relation to, 1) the amount of morphine in single or repeated administration, 2) the duration of morphine-repetition, 3) the abrupt withdrawal or nalorphine-administration and 4) the duration of abstinent days in the cases of morphine-reinjection.

MATERIALS AND METHODS

Male albino rats of Wistar Imamichi strain weighing about 100g at the beginning of experiments supplied by Central Laboratory for exp. Animals in Tokyo, were used throughout the experiments. Four or five rats were fed in one cage with dried food (CLEA No. 1) and water was taken ad libium from calibrated polyethylen bottle. Body weights of rats were measured daily between 10~12 a.m. except Sunday. Morphine hydrochloride was injected sub-

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cutaneously twice daily including Sunday usually after weighing and at 8~10 p.m. on the back of rats and the concentration of morphine solution (as free base) were modified not to exceed over 1.0 ml in their quantities. The control rats were injected 1.0 ml of physiological saline solution by the completely same procedure. For the sake of morphine withdrawal, physiological saline solution was substituted to morphine solution. For the measurement of the Sp. Act. of rats, the "electronic activity level counter by Yanagita" was used throughout the experiments (Fig. 1). As stated above, this instrument clearly shows the amounts of the Sp. Act. of small animals at every necessary intervals for necessary duration by numerical values (Fig. 2) or by the length of vertical line on kymograph-paper. One disadvantage of this instrument is that the amount of the activity shown is not an absolute but a relative value.

Fig. 1

Fig. 1 Yanagita's electronic activity level counter for small animals.

Fig. 2 Activity level is shown by number.

Fig. 2

RESULTS

I. *Changes of the Sp. Act. of rats by the administration of physiological saline solution.*

Rats were injected physiological saline solution subcutaneously twice daily

Fig. 3 Changes of the spontaneous activity of rats by the first administration of NaCl solution.

Abscissa shows time in minutes.
Vertical line shows the amount of activity by numerals.
↓ indicates injection.

Fig. 4 Changes of the Sp. Act. due to the injection of NaCl sol. on 15th day after the start of repeated injection.

for 17 days continuously and the changes of their Sp. Act. before and after the injection were recorded daily. At the beginn of the experiments, the Sp. Act. of rats showed temporarily slight increases after the injection as Fig. 3 shows. But no change was recognized any more on the 7th day and thereafter. (Fig. 4) It could be known therefore that the Sp. Act. of rats are not influenced at all by the repeated administration of physiological saline solution for 17 days except slight increases at the beginning.

II. *Changes of the Sp. Act. of rats by the administration of 5 mg/kg morphine.*

The changes of the Sp. Act. of rats to whom 5 mg/kg morphine was sub-

Fig. 5 Changes of the Sp. Act. of rats due to the first injection of 5 mg/kg morphine.

Fig. 6 Changes of the Sp. Act. of rats due to the injection of 5 mg/kg morphine on the 5th day of morphine-repetition.

Fig. 7 Changes of the Sp. Act. of rats due to the injection of 5 mg/kg morphine on the 7th day.

Fig. 8 Changes of the Sp. Act. of rats due to the injection of 5 mg/kg morphine on the 9th day of morphine-repetition.

Fig. 9 Changes of the Sp. Act. of rats due to the injection of 5 mg/kg morphine on the 15th day.

cutaneously injected twice daily for 17 days repeatedly, were recorded on 1st, 3rd, 5th, 7th, 9th and 15th day. As seen in Fig. 5 and 6, the Sp. Act. of the rats were not significantly influenced by the single administration at the beginning nor by the repeated administration for 5 successive days. However, on 7th day, by the administration of the same amount of morphine the Sp. Act. of the rats began to show some increase and on 9th day and thereafter they showed significant increase. (Figs. 7, 8 and 9).

III. Changes of the Sp. Act. of rats by the administration of 20 mg/kg morphine.

Fig. 10 shows that the Sp. Act. of rats were decreased by the first single

Fig. 10 Changes of the Sp. Act. of rats due to the first injection of 20 mg/kg morphine.

Fig. 11 Changes of the Sp. Act. of rats due to the injection of 20 mg/kg morphine on the 3rd day.

Fig. 12 Changes of the Sp. Act. of rats due to the injection of 20 mg/kg morphine on the 5th day.

Fig. 13 Changes of the Sp. Act. of rats due to the injection of 20 mg/kg morphine on the 9th day.

Fig. 14 Changes of the Sp. Act. of rats due to the injection of 20 mg/kg morphine on the 13th day.

injection of 20 mg/kg morphine. It can be seen however in Figs. 11, 12, 13 and 14 that the Sp. Act. showed no decrease any more on 3rd day and that they turned into increase on 5th day and into significant increases on 9th day and thereafter.

IV. *Changes of the Sp. Act. of rats by the administration of 50 mg/kg morphine.*

As shown in Figs. 15, 16 and 17, the Sp. Act. of the rats, after showing decrease at the first injection, were slightly increased on 5th day by the repeated administration of 50 mg/kg morphine and significantly increased on 7th day and thereafter.

Fig. 15 Changes of the Sp. Act. of rats due to the first injection of 50 mg/kg morphine.

Fig. 16 Changes of the Sp. Act. of rats due to the injection of 50 mg/kg morphine on the 5th day of morphine-repetition.

Fig. 17 Changes of the Sp. Act. of rats due to the injection of 50 mg/kg morphine on the 13th day.

Here the changes of the Sp. Act. of rats due to the repeated administration of various amounts of morphine can be summarized as table 1.

Table 1
Changes of Spontaneous Activities of Rats by the Repeated Administrations of Morphine in Various Dosis at Various Date

Dose of morphine HCl	Date when rats activity showed changes by repeated morphine injection			
	Decrease	No change	Slight increase	Significant increase
5 mg/kg twice daily		1st day	7th day	9th day
20 mg/kg twice daily	1st day	3rd day	5th day	9th day
50 mg/kg twice daily	1st day		3rd day	7th day

It can be recognized from this table that the Sp. Act. of rats become gradually to be less decreased by the repetition of morphine injection and when the morphine administration is continued further, their Sp. Act. turn into increase by and by.

V. *Changes of the Sp. Act. of morphinized rats by the single injection of smaller or bigger dose of morphine.*

As shown in Fig. 18, the Sp. Act. of rats to whom 5 mg/kg morphine had been administered repeatedly for 17 days twice daily, were increased by the single injection of 50 mg/kg morphine at first and then decreased temporarily at about 60 minutes after injection, while the Sp. Act. of rats who had been receiving 50 mg/kg morphine for 17 days twice daily, showed continuous increase after the administration of 20 mg/kg morphine. (Figs. 18 and 19)

Fig. 18 Changes of the Sp. Act. of rats who had been receiving 5 mg/kg morphine repeatedly for 17 days, by the administration of 50 mg/kg morphine.

Fig. 19 Changes of the Sp. Act. of rats who had been receiving 50 mg/kg morphine repeatedly for 17 days, by the administration of 20 mg/kg morphine.

VI. *Changes of the Sp. Act. of rats to whom increasing dose of morphine were repeatedly administered over a month period.*

After ascertaining that the injection of physiological saline solution made no significant changes upon the Sp. Act. of 4 rats (Fig. 20) and that the first injection of 20 mg/kg morphine to the same rats caused decrease of their Sp. Act. (Fig. 21), morphine was repeatedly administered twice daily for 35 days continuously to the same four rats increasing the dose every 5 days till it reached to 100 mg/kg and maintaining 100 mg/kg for 10 days.

The Sp. Act. of the rats on the 16th day and 23rd day are shown in Figs. 22 and 23 respectively. As clearly recognized in the figures, the more and the longer morphine was administered, the more significantly the increase of the Sp. Act. was observed.

Fig. 24 represents the changes of the Sp. Act. of the 2 rats among the 4

Fig. 20 Changes of the Sp. Act. of rats due to the injection of saline solution.

Fig. 21 Changes of the Sp. Act. of the same rats as in Fig. 20, due to the first injection of 20 mg/kg morphine.

Fig. 22 Changes of the Sp. Act. of the same rats as in Fig. 21, who had been receiving morphine repeatedly for 15 days, by the administration of 60 mg/kg morphine.

Fig. 23 Changes of the Sp. Act. of the same rats as in Fig. 22 who had been receiving morphine repeatedly for 22 days, by the injection of 80 mg/kg morphine.

which were injected 100 mg/kg morphine in the morning of 36th day and Fig. 25 shows those of the other 2 rats by the withdrawal of morphine at the same time.

Fig. 24 Changes of the Sp. Act. of the two of the same rats as in Fig. 23 who had been receiving morphine repeatedly for 35 days, by the administration of 100 mg/kg morphine.

Fig. 25 Changes of the Sp. Act. of the two of the same rats as in Fig. 23 who had been receiving morphine repeatedly for 35 days, by the administration of NaCl solution.

Fig. 26 Changes of the Sp. Act. of rats who had been receiving morphine repeatedly for 35 days, by the administration of 10 mg/kg nalorphine.

By comparing the two figures (24, 25) it is easily understood that the Sp. Act. of rats who had been receiving morphine repeatedly over a month were increased very markedly after the injection of morphine while the withdrawal of morphine caused significant decreases of the Sp. Act. of the similarly morphinized rats.

VII. *Changes of the Sp. Act. of morphinized rats by the administration of 10 mg/kg nalorphine.*

Fig. 26 shows the change of the Sp. Act. by the injection of 10 mg/kg nalorphine to the rats to whom morphine was repeatedly and increasingly administered twice daily for 35 days.

It is clear that the injection of 10 mg/kg nalorphine caused significant decreases of the Sp. Act. of morphinized rats.

VIII. *Changes of the Sp. Act. of the morphine-withdrawn rats by single re-injection of morphine.*

Ten rats were injected morphine twice daily for 49 days increasing the dose till 100 mg/kg every five days and maintaining the last dose for 29 days. On 50th day, physiological saline solution was substituted for morphine solution and the injection caused significant decreases of the Sp. Act. of all the rats (Fig. 27). Then, the injection of saline solution was repeated twice daily to those rats. Single reinjection of 100 mg/kg morphine was given to one of these ten morphine withdrawn rats one by one, on 5th, 6th, 9th, 10th, 12th, 15th, 20th, 28th, 30th and 48th abstinent day respectively.

As can be understood by figures; 1) the Sp. Act. of the rats were clearly increased by the single reinjection of 100 mg/kg morphine on 5th~20th day after the withdrawal. (Figs. 28, 29 and 30). 2) It was nearly a month later

Fig. 27 Changes of the Sp. Act. of rats who had been receiving morphine repeatedly for 49 days, by the injection of saline solution. Length of vertical line shows the amount of activity every five minutes.

Fig. 28 Changes of the Sp. Act. of one morphinized rat due to the reinjection of 100 mg/kg morphine on 5th abstinent day.

Fig. 29 Changes of the Sp. Act. of one morphinized rat due to the reinjection of 100 mg/kg morphine on 12th abstinent day.

Note: the difference between NaCl and morphine!

Fig. 30 Changes of the Sp. Act. of one morphinized rat due to the reinjection of 100 mg/kg morphine on 20th abstinent day.

Fig. 31 Changes of the Sp. Act. of one morphinized rat due to the reinjection of 100 mg/kg morphine on 30th abstinent day.

Fig. 32 Changes of the Sp. Act. of one morphinized rat due to the reinjection of 100 mg/kg morphine on 47th abstinent day.

after withdrawal when the reinjection of morphine caused no increase of the Sp. Act. any more (Fig. 31). 3) On the 47th abstinent day, the rat first showed a decrease of its Sp. Act. by the single reinjection of 100 mg/kg morphine (Fig. 32) as same as in the case of the first injection of 20 mg/kg morphine to non-tolerant rat.

It can be known from these results that the influence of the repeated

morphine administration upon the Sp. Act. of rats remains for a long time after morphine withdrawal.

IX. *Effects of the repeated administrations of nalorphine upon the changes of the Sp. Act. due to the single reinjection of morphine of the morphine withdrawn rats.*

Six rats had been injected morphine repeatedly for 47 days twice daily increasing the dose from 20 mg/kg to 100 mg/kg every five days and maintaining the last dose for 27 days. In the morning of 48th day, the rats were injected 10 mg/kg nalorphine in turn of morphine and their Sp. Act. were recorded (Fig. 33).

Thereafter, 10 mg/kg nalorphine were injected repeatedly 4 times daily (0.00, 7.00, 13.00, 19.00) to these rats. One by one on every successive day, one of the six rats was reinjected 100 mg/kg morphine (10.00 a.m.) 3 hours after the injection of nalorphine, and his Sp. Act. was recorded. The rat reinjected morphine was discarded after the recording. The numbers of the records were 6 from the second day to the seventh day of nalorphine injection. Figs. 34, 35 and 36 are the records of the changes of the Sp. Act. before and

Fig. 33 Changes of the Sp. Act. of one morphinized rat due to the injection of 10 mg/kg nalorphine.

Fig. 34 Changes of the Sp. Act. of the rat who had been receiving 10 mg/kg nalorphine 4 times daily for $2\frac{1}{2}$ days after 47 days of morphinization, due to the reinjection of 100 mg/kg morphine.

Fig. 35 Changes of the Sp. Act. of the rat who had been receiving 10 mg/kg nalorphine 4 times daily for $4\frac{1}{2}$ days after 47 days of morphinization, due to the reinjection of 100 mg/kg morphine.

Fig. 36 Changes of the Sp. Act. of the rat who had been receiving 10 mg/kg nalorphine 4 times daily for $6\frac{1}{2}$ days after 47 days of morphinization, due to the reinjection of 100 mg/kg morphine.

after the morphine reinjection on 3rd, 5th and 7th day of repeated nalorphine administration respectively.

It can be assumed from the results already mentioned that if nalorphine were effective for the acceleration of tolerance disappearance, frequently repeated administration of nalorphine would depress the increase of the Sp. Act. due to the reinjection of morphine in once-morphinized rats.

As the figures show, however, it was not the case. It is most likely therefore, that the repeated administration of nalorphine do not affect the duration of tolerance already formed in rats body.

DISCUSSIONS

It is generally accepted that the development of tolerance to the various effects of morphine are not uniform, some effects are tolerated very fastly, some slowly and some never. The disappearance of narcotic effects is the usual criterion for the development of tolerance to morphine. And the changes of the spontaneous activity are one of the narcotic effects on rats apparently modified by the repeated administration of morphine.

Kaymakcaran and Woods⁽⁷⁾ (1956) stated, "during chronic administration of morphine the most apparent sign of tolerance development was the disappearance of sedation which follows the administration of morphine in non-tolerant rats. Indeed instead of sadation the rats exhibited more and more central stimulation after the morphine injection."

Results of our experiments I~IV show the reliability of their statement *by number* although they did not mention the correlation between the development of tolerance and the size of the dose, the number of the days of morphine administration, etc.

Table I discloses these points clearly as follows; 1) The more the amount of repeatedly administered morphine is large, the faster the tolerance developes. This is in accordance with the results by Schmidt and Livingston⁽²⁾ (1933), Eddy and Reid⁽⁸⁾ (1934) got in dogs. 2) The development of tolerance to the effects of morphine upon the Sp. Act. of rats was recognized as early as in 2~3 days of repeated administration, i.e., the sedative effect of morphine which had been observed on 1st day of 20 mg/kg morphine administration was not manifested in the same rats any more on 3rd day of morphine repetition and in the case of 50 mg/kg morphine the tendency to increase their activity was recognized as early as on 3rd day.

Wikler and Carter⁽⁹⁾ (1953), based on their careful observations, insisted that abstinent changes could be precipitated by nalorphine before the develop-

ment of tolerance which were demonstrated by the hindlimb reflexes in chronic spinal dogs.

However, as stated at the beginning of this discussion, the manifestations of various morphine actions are not depressed uniformly by the repeated administration of morphine. So, unless the tolerance to the hindlimb reflexes are proved to occur earlier than the changes of the Sp. Act. and to occur earliest among many other manifestations of morphine actions, their conclusion can hardly be accepted.

Authors had once reported in their studies on N-allyl-normorphine; 1) morphinized rats showed without fail sharp decreases of their body weights temporarily after the abrupt withdrawal of morphine or the injection of nalorphine but no decrease after morphine injection and therefore, such decreases of the body weights could be regarded as one of the signs of physical dependence formed by the repeated morphine administration. 2) the decrease of the body weights of morphinized rats due to 10 mg/kg nalorphine could be scarcely recognized on 2nd~4th day after the start of repeated morphine administration and was recognized significantly on 6th day, though nalorphine itself did not affect the bodyweights of non-tolerant rats.

Therefore, it may be said that the appearance of tolerance in rats can be recognized no later than the formation of physical dependence in so far as the Sp. Act. by Yanagita's activity level counter is adopted for the measurement of tolerance development.

The authors accordingly are inclined to assume that some parts of tolerance to morphine develop fast in rats as physical dependence does so.

It is understandable from the results of Exp. I~IV and Exp. V that the tolerance is not formed to the excitatory effects of morphine but to the sedative effects as ascertained by former worker (Gold⁽¹⁰⁾ 1929).

However, the extraordinary increase of the Sp. Act. due to the repeated administration of morphine for 36 days shown in Fig. 24 cause some doubt that the excitatory effects might be rather accelerated or accumulated by the repetition of morphine administration. Indeed, it is difficult for the authors to understand that the remarkable differences of the activities of the same rat between in Fig. 21 and Fig. 24 depend solely upon the decrease of the sedative effect, though some people are satisfied with the explanation that the increased susceptibility to the convulsion does develop. Further studies seem to be necessary on this point.

As to the changes of the activities of morphinized rats by the abrupt

withdrawal of morphine or by the administration of nalorphine, conflicting results were reported. Ma⁽¹¹⁾ regarded "depressive state" as a sign of the abstinent phenomena of rats. Kaymakcaran and Woods observed sedation of morphinized rats after the administration of nalorphine. Lately on the other hand, Hanna⁽¹²⁾ (1960) and Gunne⁽¹³⁾ (1961) reported the marked hyperirritability in the severely morphinized rats by the administration of nalorphine.

According to the present authors experiences, the way of rapid morphinization of rats by Hanna caused relatively high mortality during the frequent administration of large dose of morphine (nine deaths out of 36 rats) and also by the administration of nalorphine (4 deaths out of 11 rats) while no death was observed in the slow morphinization by Kaymakcaran and Woods throughout many experiments.

Joël and Ettinger⁽¹⁴⁾ (1926) had already mentioned "Kachexie und Abmagerung" by the repeated administration of large dose of morphine. And Schmidt and Livingston (1933) reported that too rapid increase of the dose of morphine inhibited the development of tolerance by the toxic side effects and that the tolerance already developed was partially or completely abolished again by overdosage.

These were the reasons why the present authors chose the way of "slow morphinization."

The results of Exp. VI and VII stand on the side of Ma, Kaymakcaran and Woods, showing *by number* the occurrence of sedation instead of excitation in morphinized rats by the withdrawal of morphine or by the administration of nalorphine.

As to the disappearance of tolerance after the cessation of morphine administration too, there were some contradictory reports.

Joël and Ettinger claimed that tolerance was lost less rapidly than it was aquired and they could detect difference three weeks after cessation whereas Reynolds and Randall stated in their book (1957) "tolerance disappears within a few days to two weeks following cessation and the characteristic depressant effect can again be elicited with small dose."

Of course, species difference also must be brought into consideration here.

Although the results of our Exp. VIII can not be treated statistically because of the shortage of samples, they suggest that tolerance or the reaction of morphinized rats increasing their Sp. Act. by morphine reinjection, remains far longer time after cessation than it was believed by Reynolds and Randall. And this may be one of the reasons why reinjection of morphine seems to develop tolerance more easily and earlier in once-tolerant animal than in non-tolerant animals even

after many days of cessation of medication.

One must be careful therefore, not to reuse the once-tolerant rats early after cessation of morphine administration.

As shown in Exp. VIII, tolerance or the phenomena recognized by the increase of the Sp. Act. after morphine reinjection, seems to be retained in rats body for a long time .

This is in accordance with the result by Plant and Pierce⁽¹⁵⁾ (1928) who proved the long lasting of the tolerance to the emetic effect of morphine on dogs.

Although Seevers had showed that nalorphine were worthless in accelerating the disappearance of tolerance in monkeys, the doubt whether or not the continuous administration of nalorphine to the morphine-withdrawn rats might shorten the duration of tolerance retention in rats body, have been kept in authors mind. Because, since the antagonistic effect of nalorphine is assumed to be related to its competition with morphine at the same effector sites of nerve cells, continuous supply of nalorphine might keep more morphine free from binding and consequently more morphine being forced to leave from the receptor might be excreted fast and finally the tolerance might disappear earlier.

The results of Exp. IX reconfirmed the statement of Seevers, showing that the hyperactivity of the morphine withdrawn rats due to the reinjection of morphine was not influenced at all by the injection of 10 mg/kg nalorphine 4 times daily for 7 successive days.

It could be known therefore that the frequent administrations of nalorphine do not affect the tolerance already established in rats.

SUMMARY

Using Yanagita's activity-level counter, effects of morphine administration upon the spontaneous activities of rats under various conditions were studied. And the following facts were elucidated;

1. It was shown *numerically* that the more the size of the dose was large and the more the duration of morphine repetition was long, the faster and the stronger their spontaneous activities became.
2. Such changes of the spontaneous activities of rats due to the repeated administration of morphine can be regarded as one the signs of tolerance.
3. The changes of the spontaneous activities of rats or some sorts of tolerance occur as fast as the appearance of physical dependence.
4. The mechanisms by which rats become hyperactive by the repeated administration of morphine may be explained chiefly by the development of tolerance to the sedative action of morphine and by the absence of tolerance to

the excitatory action of the drug. However, the extraordinary increases recognized by the long term repetition of morphine request us further studies.

5. As far as slow morphinization of rats is adopted, the abstinent phenomenon of the morphinized rats is sedation.

6. The disappearance of tolerance after the cessation of morphine administration occurs very late, far later than the disappearance of abstinent phenomenon.

7. Frequent administrations of nalorphine to the morphinized rats can not shorten the duration of tolerance retention after the cessation of the medication.

REFERENCES

1. Eddy, N. B.: The pharmacology of opium alkaloids U. S. P. H. S. Suppl. 163, p. 711, 1941.
2. Schmidt, C. F. and Livingston, A. E.: J. P. E. T. 47: 443, 1933.
3. Reynolds, A. K. and Randall, L. O.: Morphine and allied drugs, p. 21-25, Univ. Tronto Press, 1957.
4. Schauman, O.: Morphine u. morphineähnlich wirkende Verbindungen, p. 81-84, Springer Verlag 1957.
5. Seevers, M. H.: Fed. Proc. 13: 672, 1954.
6. Nakao, T. and Yanagita, T.: Jikeikai Med. J. 5: 134, 1958.
7. Kaymakcaran, S. and Woods, L. A.: J. P. E. T. 117: 112, 1956.
8. Eddy, N. B. and Reid, J. P. E. T. 52: 468, 1934.
9. Wikler, A. and Carter, R. L.: J. P. E. T. 109: 92, 1953.
10. Gold, H.: J. P. E. T. 35: 355, 1929.
11. Ma, W. C.: Chinese J. Physiol. 45: 1200, 1931.
12. Hanna, C.: Arch. intern. pharmacodyn. 124: 326, 1960.
13. Gunne, L. M.: Psychopharmacologia 2: 214, 1961.
14. Joël, E. and Ettinger, A.: Arch. exp. Path. u. Pharm. 115: 334, 1926.
15. Plant, O. H. and Pierce, I. H.: J. P. E. T. 33: 329, 1928.