for its virulence and fulminating course. All organ systems are involved, more or less, by the occurrence of widespread petechial hemorrhages and/or septic thrombi, as well as disseminated miliary abscesses. In frogs the skin of the legs becomes markedly hyperemic and hemorrhagic. Death is the usual termination of the disease.

During the course of studies in this laboratory on *Bufo marinus*, three specimens of native toads (*B. boreas* and *B. woodhousii*) were introduced into a colony of 30 specimens of *B. marinus* that had been in excellent health in the laboratory for 16 months. Within 10 days the first case of an epidemic disease, which destroyed a third of the colony before it was controlled, appeared. The disease was studied bacteriologically[2] and pathologically and found to be a classical example of *F. hydrophila* septicemia. Source of the infection was subsequently traced to an infected frog colony with which the native toads had been housed prior to their acquisition by the author.

In view of the reports that chloromycetin (chloramphenicol) is effective *in vitro* (*2*) against various species of *Proteus* and *Pseudomonas* and in the treatment of human urinary tract infections (*4*), this drug was tried on the remaining animals in the colony. At the beginning of treatment most of the animals showed the early stigmata of the disease, namely, moderate reddening and capillary dilatation over the lower belly. The dosage was derived empirically from that used in man and other mammals, and set at 5 mg/100 g initially, followed by 3 mg/100 g twice daily for five days. The drug was dissolved in distilled water in concentrations of 5 mg/ml for the initial dose and 3 mg/ml for subsequent doses. Solution of these amounts was readily accomplished (in contradiction to the solubility data given by Woodward [*10*] by slight warming. The solutions were administered by gastric intubation, a procedure which is easily performed in these animals.

One of the treated animals was in an advanced stage of the disease (signs of extensive central nervous system damage) at the beginning of therapy. This animal survived 4 more days (twice the survival expectancy of untreated animals in advanced stages) but succumbed to what superficially resembled myocardial decompensation in mammals, presumably due to irreversible myocardial damage incurred before inception of therapy. The remainder of the animals responded well. Now, some two months later, they have shown no further evidence of the disease and again are in their former state of excellent health.

These results suggest that colonies of amphibians may be maintained free from infection by *P. hydrophila* by the administration of chloromycetin at the time the colony is established, either in the water in which the animals are kept, or, preferably, in the manner described above. Similar measures might be practiced on new additions to the colony. The latter seems desirable in view of the report by Emerson and Norris (*5*) that the disease is endemic in many of the natural habitats from which frogs are collected commercially.

[2] Thanks are due Elizabeth O'Toole, of the Department of Bacteriology of the University of Colorado School of Medicine, with whom a detailed report of the pathology and bacteriology of the infection in *B. marinus* will be published elsewhere.

References

1. BERGEY, D. H., *et al.* *Manual of determinative bacteriology* (5th ed.). Baltimore: Williams & Wilkins, 1939.
2. BLISS, E. A., and TODD, H. P. *J. Bact.*, 1949, **58**, 61.
3. BREED, R. S., MURRAY, E. G. D., and HITCHENS, A. P. *Bergey's manual of determinative bacteriology* (6th ed.). Baltimore: Williams & Wilkins, 1948.
4. CHITTENDEN, G. E., *et al.* *J. clin. Invest.*, 1949, **28**, 1052.
5. EMERSON, H., and NORRIS, C. *J. exp. Med.*, 1905, **7**, 32.
6. ERNST, P. *Beitr. Path. Anat. Allgem. Path.*, 1890, **8**, 203.
7. KULP, W. L., and BORDEN, D. G. *J. Bact.*, 1942, **44**, 673.
8. RUSSELL, F. H. *J. A. M. A.*, 1898, **30**, 1442.
9. SANARELLI, G. *Zentr. Bakt. Parasitenk., Orig.*, 1891, **9**, 193, 222.
10. WOODWARD, T. E. *Ann. int. Med.*, 1949, **31**, 53.

A Diploid Form of *Medicago sativa* L.

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There have been numerous cytological investigations of common alfalfa, *Medicago sativa* L. Reviews by Fryer (*2*) and Senn (*4*) show that the normal chromosome number in somatic tissues is 32. Fryer reported one exceptional plant as having 35 chromosomes. Other abnormal numbers were 31 and 33, found by Skovsted (*5*) in a study of twin seedlings. The work of Ledingham (*3*) strongly suggests that the species originated as an autotetraploid, and Tysdal *et al.* (*7*) have concluded that at least certain genetic data could be interpreted on the basis of tetrasomic ratios rather than the commonly used disomic approach. If *M. sativa* has an autotetraploid origin, it is to be expected that fertile 16-chromosome forms would exist. So far as the authors are aware, no such forms have been reported. It is the purpose of this paper to record what appears to be a 16-chromosome form of *M. sativa* and to present preliminary data on its cytology and breeding behavior.

In 1947 a small sample of seed labeled *Medicago sativa* was received from Russia through H. A. Senn, senior botanist, Division of Botany and Plant Pathology, Science Service, Ottawa. It was given the Saskatoon accession number S-2128. According to information accompanying the sample, it came from the Botanical Gardens, Academy of Sciences, Armenian S.S.R. at Erevan, Kanaku, U.S.S.R., and was collected from one wild-growing plant. Forty-three seedlings were established in the field nursery at Saskatoon in June, 1949.

The plants started to flower in early August, and it was then apparent that S-2128 was not a normal form of *M. sativa*. It was similar to the latter in having purple flower color, with no trace of yellow present. It was similar also in that a later examination showed the pods to have up to 3 or 4 coils and the relative lack of pubescence common to *M. sativa*. In various characters, how-

ever, such as size of flowers, pods, seeds, leaflets, and fineness of stem, it was much more like 16-chromosome forms of *M. falcata* than normal *M. sativa*. It exhibited also the decumbent growth habit common to 16-chromosome forms of *M. falcata*.

Root-tip smears were made from several plants of S-2128 and were compared to similar material from typical plants of *M. sativa*. The technique followed was based upon that described by Smith (*6*). Living root tips were first placed in a saturated solution of paradichlorobenzene for 30–60 min to shorten the chromosomes before being killed and were left for 24 hr in a 3:1 solution of 95% ethyl alcohol and glacial acetic acid. They were then softened for 3–5 min in a 1:1 solution of 95% ethyl alcohol and concentrated HCl, and returned to the alcohol-acetic acid solution for at least 3 min before staining with acetocarmine.

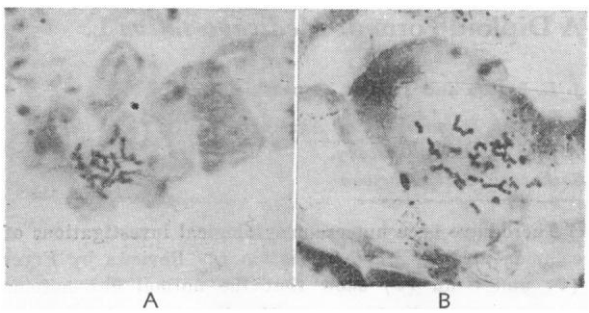

FIG. 1. *A*, somatic chromosomes from S-2128, magnification approximately × 1,300. *B*, somatic chromosomes from typical plant of *M. sativa*, approximately × 1,300.

Fig. 1 *A* is representative of a large number of determinations from root-tip cells of S-2128. The chromosome number is definitely 16. Cell size and chromosome length were not determined, and any apparent differences in length of the chromosomes, based upon *A* or *B* in Fig. 1, would not necessarily represent true differences, because the two preparations were not consistently left in the shortening solution for exactly the same duration. Even if this had been the case, it is possible that the cells of different root tips might react unequally to the paradichlorobenzene. In his study of *M. sativa* and *M. media*, Fryer (*2*) did not observe chromosomes with satellites. In Fig. 1 *B* there are four chromosomes, each with what appears to be a satellite. Similar formations involving two chromosomes have been observed in S-2128 and show faintly at the lower left-hand corner of the field in Fig. 1 *A*. Although these structures have been noted in other cells, it is felt that further studies including meiotic divisions are necessary in order to establish definitely the presence of satellites. If these observations are confirmed they would afford good supporting evidence for the autotetraploid origin of *M. sativa*.

In addition to the cytological data presented above, data were obtained also from a series of crosses involving S-2128 as the pistillate parent. All crosses were made August 11 and harvested September 17. Ordinarily the time allowed was sufficient for seed to mature. The period was near the end of the normal seed-setting season, however, and temperatures were lower than usual. As a result, some of the seed harvested, particularly from selfing and intercrossing, was not fully matured. The flowers were not emasculated for selfing or intercrossing. All flowers outcrossed were emasculated by clipping the standard, tripping the staminal column, and then blowing off the pollen. The data obtained are summarized in Table 1.

TABLE 1

SUMMARY OF SELFING AND CROSSING RESULTS

Parents	No. of flowers pollinated	No. of pods set	No. of seeds	Seeds per flower	Seeds per pod
S-2128	226	20	25	0.11	1.25
S-2128 intercrossed	341	275	1278	3.75	4.65
S-2128 × *M. sativa* (32-chromosome form)	149	45	21	0.14	0.47
S-2128 × *M. falcata* (32-chromosome form)	53	21	6	0.11	0.30
S-2128 × *M. falcata* (16-chromosome form)	147	115	603	4.10	5.24

The selfing results suggest that S-2128 is rather highly self-sterile. The 226 flowers self-pollinated (Table 1) represented 11 plants. The 20 pods and 25 seeds harvested represented only 120 flowers from 4 plants, however. The remaining 106 flowers from 7 other plants proved to be completely self-sterile. The intercrosses show fairly normal cross-fertility, as compared with the average of 5.54 seeds per pod found previously (*1*) in a study of 32-chromosome *M. sativa*. Bolton (*1*) has shown that intercrosses between related plants set fewer seeds per flower, and that the progenies of these crosses are lower in seed and forage yield than comparable outcrosses. As previously noted, the information accompanying the original sample of S-2128 stated that the seed had been collected from one plant. That statement is supported by the results of selfing and intercrossing, as well as by the relative uniformity of the progeny for morphological characters.

When S-2128 was outcrossed to 32-chromosome forms, the results were similar whether *M. sativa* or *M. falcata* was the staminate parent. The number of seeds per flower was similar to that from selfing, but many of the pods contained only aborted seeds, which were not included in the calculations of total seeds harvested. These results compare closely with unpublished results obtained at Saskatoon, where it was found that normal seed-setting occurred in crosses between 32-chromosome forms of *M. falcata* and *M. sativa*, but that, when pollen from 32-chromosome *M. sativa* was applied to the stigmas of 16-chromosome *M. falcata*, although a fair percentage of the crossed flowers set pods, the pods contained only aborted seeds. The few seeds obtained from S-2128 × 32-chromosome forms have not been tested. They may prove to be selfs, although this is unlikely, both because the flowers were emasculated, and because some of the plants involved set no seed when selfed. It is more likely that they will prove to be triploids or induced tetraploids, such as those reported by Ledingham (*3*) in crosses between

16-chromosome *M. falcata* and 32-chromosome *M. sativa*.

To summarize, it may be said that a 16-chromosome form of *M. sativa* has been found. This form is highly self-sterile and highly cross-sterile when crossed to 32-chromosome forms of *M. sativa* and *M. falcata*. It shows normal cross-fertility when crossed to 16-chromosome forms of *M. falcata*.

References

1. BOLTON, J. L. *Sci. Agric.*, 1948, **28**, 97.
2. FRYER, J. R. *Canad. J. Res.*, 1930, **3**, 3.
3. LEDINGHAM, G. F. *Genetics*, 1940, **25**, 1.
4. SENN, H. A. *Bibliogr. Genet.*, 1938, **12**, 175.
5. SKOVSTED, A. *Compt. rend. Ser. Physiol.*, 1939, **22**, 427.
6. SMITH, L. *Stain Tech.*, 1947, **22**, 17.
7. TYSDAL, H. M., KIESSELBACH, T. A., and WESTOVER, H. L. *Neb. Exp. Sta. Res. Bull.*, 1942, 124.

Quantitative Aspects of the Action of Insulin on the Glucose and Potassium Metabolism of the Isolated Rat Diaphragm

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In a previous communication (*3*) experiments were described from which it was concluded that the utilization of glucose by the isolated rat diaphragm is associated with a shift of potassium from the medium into the tissue, and that both glucose utilization and potassium shift are increased by the addition of insulin. In order to study the quantitative aspects of these reactions, experiments were carried out with varying concentrations of insulin.

The technique was different from that used previously. Rats of 80–100-g body weight were decapitated after fasting for 24 hr; their diaphragms were removed and cut into quarters. The quarters were kept in ice-cold buffer solution (*2*) before the actual experiment started. Eight quarter-diaphragms, representing one-half of the left and right hemidiaphragms of 4 rats, were transferred to a flask containing 2 ml of buffer solution in which 200 mg % of glucose had been dissolved. After equilibration with a gas mixture containing 93% O_2 and 7% CO_2, the buffer-diaphragm system was incubated for 1 hr at 37° C, with shaking at a rate of 120/min. The remaining 8 quarter-diaphragms of the same rats were incubated in a buffer-glucose solution of the same composition that contained, in addition, insulin in the concentration to be tested.

This arrangement was chosen so as to make the conditions under which the diaphragms were incubated as similar as possible, with the only difference that insulin was present in one flask and absent from the control flask.

[1] Holding a grant from the Organon Laboratories, Inc., Oss, Netherlands.

After 1 hr the flasks were cooled and the contents centrifuged. Glucose (*5*) and potassium (*7*) concentrations in the medium were determined, and the diaphragms were weighed after blotting on filter paper. The difference (Δ glucose) between the quantities of glucose (calculated as mg/100 mg wet tissue) that have disappeared from the medium in the flask with insulin and that without insulin is the effect of the added insulin on glucose utilization.

While studying the changes in potassium content of the medium in these incubation experiments, it was found that, with the use of quarter-diaphragms, the potassium content of the medium increased as a rule, whereas it usually decreased when hemidiaphragms were used. Apparently a diffusion of potassium out of the "surviving" tissue takes place more rapidly from quarter-diaphragms than from hemidiaphragms. This is easily understandable because of the greater damage to the tissue that takes place when the diaphragm is divided into 4 parts.

In the present series of experiments the potassium content of the medium increased in both flasks, but in the vessel containing insulin the increase was invariably less than in the one without insulin. The difference (ΔK) between the increase of the potassium content (calculated as microequivalents per 100 mg wet tissue) of the medium in the flask with insulin and that without insulin is the effect of the added insulin on the potassium shift, associated with increased utilization of glucose by the tissue.

The glucose and potassium effects of increasing concentrations of insulin were plotted in a curve (Fig. 1). The

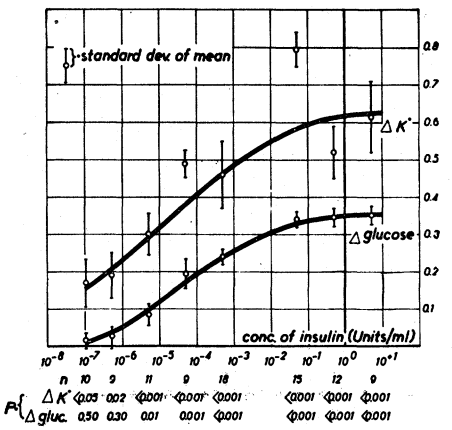

FIG. 1. Effect of varying concentrations of insulin upon glucose utilization (mg/100 mg wet tissue) and potassium shift (microequivalents/100 mg wet tissue) in experiments with isolated rat diaphragms; n = number of experiments; P = Fisher's value of probability.

graph illustrates that, with augmenting concentrations of insulin, both glucose and potassium effects increased until, at a level of about 10^{-2}–10^{1} units/ml, both effects tended to become more or less constant. In the region of lower concentrations of insulin, it was found that 10^{-7} units/ml still had a significant effect upon the potassium shift, whereas the effect upon the glucose utilization was no longer detectable. In general, the figures for the effect of insulin on potassium shift were more irregular than