velocity" refers to the fact that the flow is laminar, hence there is a velocity distribution of which the average is measured.)

The latter measurement system is applicable to instantaneous velocity measurements; that is, one would be able to measure flow between heart beats as well as during a heart beat, provided that the distance $x$ is chosen small relative to the total distance of fluid travel between heart pumps.

There is no problem of obtaining magnetic field homogeneity over the region of a human finger, but there is some difficulty if measurements are contemplated in a large region such as the human neck. We are planning to measure absolute blood velocities in human fingers as our next project.

It is difficult to attempt a comparison of different flow velocity systems at this stage of development, but in our opinion the nuclear resonance measurement system has potentialities unequaled by any other method. In essence, an NMR measurement implants disturbed nuclei whose path may be traced for any length of time up to the relaxation time $T_1$. For mouse blood, $T_1$ is approximately 0.4 sec. Human blood has a relaxation time of the same order of magnitude.

One additional important set of experimental possibilities should be mentioned. One may use nuclear or *electron* magnetic resonance as a tracer detection system. For example, consider the digestion or injection of substances containing nickel, cobalt, iron, or other transition metal salts. These may be detected in the bloodstream in vivo by means of electron paramagnetic resonance.

Detection of as little as one billionth of a gram of paramagnetic material has been achieved; however, it is expected that R-F losses due to surrounding body tissues will reduce the sensitivity somewhat. Nuclear resonance techniques may also be utilized in conjunction with specific tracer substances—almost every chemical has its own specific resonance spectrum and a host of materials would serve as tracers (*4*).

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### References and Notes

1. F. Bloch, *Phys. Rev.* **70**, 460 (1946); ———, W. W. Hansen, M. Packard, *ibid.* **70**, 474 (1946).
2. N. Bloembergen, E. M. Purcell, R. V. Pound, *ibid.* **73**, 697 (1948).
3. E. Hahn, *ibid.* **80**, 580 (1950).
4. It is a pleasure to acknowledge numerous stimulating conversations with Professors Enoch Callaway III and Robert E. Harris which initiated my interest in biological measurements.

12 June 1959

## Stimulation of Amino Acid Transport in Isolated Diaphragm by Growth Hormone Added in vitro

*Abstract.* The influence of the pituitary on the transport of α-aminoisobutyric acid-1-$C^{14}$ into the cells of isolated rat diaphragm was investigated. Hypophysectomy results in a lower-than-normal rate of α-aminoisobutyric acid-1-$C^{14}$ penetration into muscle. Adding either simian or bovine growth hormone preparations to the incubation medium in concentrations of 2.5 to 25 μg/ml of medium resulted in a doubling of the α-aminoisobutyric acid-1-$C^{14}$ penetration rate. The stimulatory effect was minimized when the hormone concentration was reduced to 0.25 μg/ml of medium.

The incorporation of leucine-2-$C^{14}$ into the protein of isolated rat diaphragm is markedly reduced by hypophysectomy and stimulated by chronic treatment with bovine growth hormone (*1, 2*) or by the addition of simian growth hormone to the incubation medium in low concentrations (*3*). Although there are many possible explanations for these pituitary effects on amino acid incorporation, it is conceivable that the effects could result, in part, from alterations in the rate of entry of amino acids into the intracellular amino acid pool. The experiment of Noall *et al.* (*4*), in which an acute injection of growth hormone enhanced the cellular concentration of the nonutilizable amino acid, α-aminoisobutyric acid (AIB), suggested that growth hormone might exert an influence on amino acid transport. The present investigation was undertaken to determine the effects of hypophysectomy and growth hormone, added in vitro, on the rate of penetration of AIB-1-$C^{14}$ into the cells of the isolated diaphragm.

Fed female rats of the Charles River strain weighing 60 to 80 gm were used in all experiments. The rats employed in the first series of experiments (Fig. 1) were hypophysectomized 40 days before sacrifice, while those used in the second series (Fig. 2) were hypophysectomized 14 days before the experiment. The method used to measure AIB-1-$C^{14}$ transport was a modification of the procedure described by Kipnis and Noall (*5*). The animals were killed by a blow on the neck, and the diaphragms were rapidly prepared according to the method of Kipnis and Cori (*6*). The "intact" diaphragm preparation was blotted on filter paper and transferred to 125-ml flasks containing 30 ml of Krebs bicarbonate buffer, *p*H 7.4. Glucose was added to the buffer at a concentration of 0.01 mole/lit. and AIB-1-$C^{14}$ at a concentration of 0.05 mmole/lit. (25,000 count/min × ml of medium). The AIB-1-$C^{14}$ was obtained from Isotope Specialties Co., Inc., at a specific activity of 1 mc/mmole. Growth hormone preparations of simian (M425B) and bovine (NIH-BGH-1) origins were dissolved in 0.15 ml of distilled water at *p*H 8 and added to the medium immediately prior to the beginning of an experiment. An equivalent amount of distilled water at *p*H 8 was added to the control flasks. The medium in the flasks was preheated to 37°C, so that the timing of the incubation period commenced with the addition of the diaphragm preparation to the flask. Immediately after the addition of the tissue, the flask was gassed with 95 percent $O_2$–5 percent $CO_2$ for 5 minutes and then sealed. Incubation was carried out with shaking (100 cy/min) for various periods up to 2 hours. At the end of the incubation period, the diaphragm muscle was dissected away from ribs and adhering tissue, washed for 10 seconds in two changes of buffer, weighed, and homogenized in 1.0 ml of 0.008*N* acetic acid. The homogenate was centrifuged, and the supernatant was diluted 1:1 with 1 percent sucrose. Aliquots of the diluted supernatant were plated at infinite thinness on aluminum planchets and after drying were counted with a gas-flow, thin end-window counter.

The results are presented as the ratio

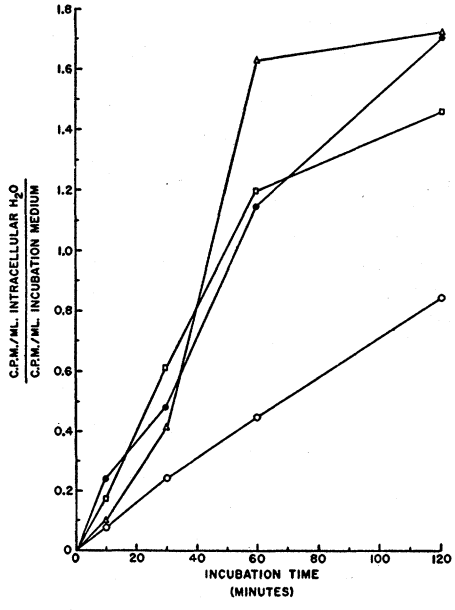


Fig. 1. Penetration of AIB-1-$C^{14}$ into "intact" rat diaphragm preparations. ●, normal; ○, hypophysectomized; △, hypophysectomized + simian growth hormone added to medium (25 μg/ml); □, hypophysectomized + bovine growth hormone added to medium (25 μg/ml). Each point represents one observation, except for the hypophysectomized controls, which represent the mean of three observations per point.

of counts per minute per milliliter of intracellular water to counts per minute per milliliter of incubation medium. The activity of intracellular water was determined by subtracting the radioactivity in the extracellular volume from that in the total tissue water. Total tissue water and extracellular volume were determined under incubation conditions similar to those used in the AIB-1-$C^{14}$ experiments. Total tissue water was obtained by drying diaphragms to constant weight at 105°C, and extracellular volume was obtained by isotope dilution, employing sucrose uniformly labeled with $C^{14}$. Sucrose-$C^{14}$ distribution reached an apparent equilibrium after approximately 15 minutes of incubation, but increased about 2 percent between 1 and 2 hours of incubation. After 2 hours of incubation, the sucrose-$C^{14}$ space of normal tissue was found to be slightly higher (19.3 percent) than that of muscle of hypophysectomized rats (17.4 percent). The addition of growth hormone preparations to the medium did not markedly alter the magnitude of the sucrose-$C^{14}$ space. In some experiments, amino acid nitrogen determinations (*7*) were made on aliquots of the medium following incubation to ascertain the amount of amino acid nitrogen released by the tissue.

The results are given in Figs. 1 and 2, in which the penetration of AIB-1-$C^{14}$ into muscle is plotted against incubation time. AIB-1-$C^{14}$ enters muscle cells of hypophysectomized rats at a lower-than-normal rate (Fig. 1). Adding either simian or bovine growth hormone preparations to the medium at a concentration of 25 $\mu$g/ml greatly enhanced the uptake of AIB-1-$C^{14}$ by the diaphragms of hypophysectomized rats. The fact that both simian and bovine growth hormones stimulated entry of AIB-1-$C^{14}$ into islolated diaphragm was of particular interest, since only simian growth hormone was found to consistently stimulate leucine-2-$C^{14}$ incorporation into diaphragm protein when added in vitro (*2*). When the concentration of the hormone preparations was reduced to 2.5 $\mu$g/ml of medium there was still a doubling in the rate of AIB-1-$C^{14}$ penetration (Fig. 2). However, a further 10-fold reduction in hormone concentration nearly eliminated the stimulatory effect. The greater rate of AIB-1-$C^{14}$ penetration in the hypophysectomized controls in the second series of experiments (Fig. 2) may be related to the shorter time (14 days) that they were hypophysectomized.

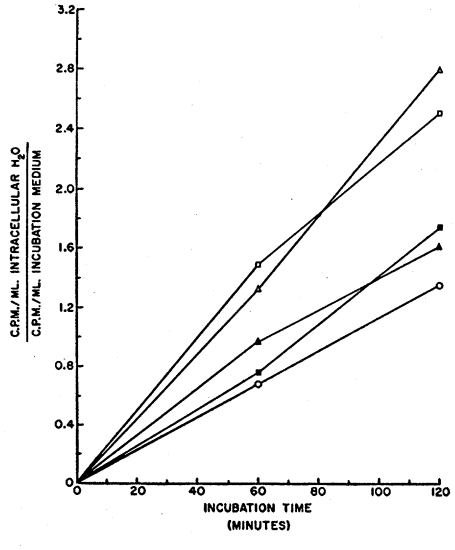


Fig. 2. Effect of low in vitro concentrations of growth harmone preparations on the penetration of AIB-1-$C^{14}$ into "intact" diaphragms of hypophysectomized rats. ○, hypophysectomized control; △, simian growth hormone (2.5 $\mu$g/ml); ▲, simian growth hormone (0.25 $\mu$g/ml); □, bovine growth hormone (2.5 $\mu$g/ml); ■, bovine growth hormone (0.25 $\mu$g/ml). Each point represents one observation.

Measurements of the amounts of amino acid nitrogen released into the medium during these experiments indicated that on a tissue-weight basis, the "intact" diaphragm preparation of a normal rat released somewhat more amino acid nitrogen into the medium than did that of a hypophysectomized animal, confirming the observations of Kline (*8*). The addition of growth hormone preparations to the medium had no effect on the rate of release of amino acid nitrogen. Since the amounts of tissue used in most experiments were approximately equivalent, the concentration of amino acid nitrogen in the medium at any time during the incubation was roughly comparable between experimentals and controls, differing only rarely by as much as 5 $\mu$g/ml of medium. Thus, the hormonally induced differences in AIB-1-$C^{14}$ transport noted in these experiments are probably not ascribable to changes in the concentration of nonlabeled, endogenously produced amino acids.

The experiments presented here support the hypothesis that pituitary growth hormone plays a role in the regulation of amino acid transport into muscle cells. Should growth hormone be found to stimulate the transport of natural amino acids as well, then the increase in amino acid incorporation into protein produced by this hormone might well be due, in part at least, to an initial increase in the availability of amino acid (*9*).

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**References and Notes**

1. J. L. Kostyo and E. Knobil, *Federation Proc.* **18,** 84 (1959).
2. ———, *Endocrinology* **65,** 395 (1959).
3. ———, *ibid.* **65,** 525 (1959).
4. M. W. Noall, T. R. Riggs, L. M. Walker, H. N. Christensen, *Science* **126,** 1002 (1957).
5. D. M. Kipnis and M. W. Noall, *Biochim. et Biophys. Acta* **28,** 226 (1958).
6. D. M. Kipnis and C. F. Cori, *J. Biol. Chem.* **224,** 681 (1957).
7. E. G. Frame, J. A. Russell, A. E. Wilhelmi, *ibid.* **149,** 255 (1943); J. A. Russell, *ibid.* **156,** 467 (1944).
8. D. L. Kline, *Endocrinology* **45,** 596 (1949).
9. We wish to express our gratitude to A. E. Wilhelmi for generously supplying the simian growth hormone used in these experiments. The bovine growth hormone was a gift of the endocrinology study section of the National Institutes of Health. This study was supported by a grant from the American Cancer Society (P-139A).
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## Stimulation of Striga asiatica (Witchweed) Seed Germination by 6-Substituted Purines

*Abstract.* Kinetin [6-(2-furfuryl)aminopurine] and certain other 6-substituted aminopurines stimulated germination of seed of *Striga asiatica* (L.) Kuntze. Optimum concentration for most active compounds was in the range of 5 to 25 mg/lit. Derivatives which showed high activity possessed an adenine nucleus with a phenyl, benzyl, phenethyl, or furfuryl radical substituted on the amino group.

*Striga asiatica* (L.) Kuntze, an angiospermous root parasite indigenous to several tropical and subtropical areas of the Eastern Hemisphere, was discovered in the coastal plain section of North Carolina and South Carolina in 1956. This species threatens warm-season gramineous crops in the infested regions. Consequently, attention is being focused for the first time in the United States on problems associated with the growth, development, and control of this particular plant parasite.

Germination of *Striga* seed depends upon a substance (or substances) secreted by the roots of host and certain other plant species (*1*). The natural germination stimulant of *Striga* spp. has not been identified. However, a sugar, D-xylulose, was reported to be very effective in promoting germination of seed of *S. hermonthica* (Del.) Benth. but did not appear to be a constituent of the root exudate of *Sorghum vulgare* (*2*). In conjunction with efforts to isolate and characterize the chemical stimulant, or stimulants, in root exudates of seedlings of corn, *Zea mays* L., various organic and inorganic compounds were tested for their ability to induce seed germination. In preliminary tests 6-(2-furfuryl)aminopurine