centrifugation. The biological activity per unit weight of virus protein in this suspension was assayed on bean plants (*Phaseolus vulgaris* L. var. Early Golden Cluster) by the local lesion method, as modified by Spencer and Price.[2] The results were as follows: The content of virus protein in the inoculated leaf continued to increase for as long as 20 days after inoculation. This was a point of immediate interest, as it had been generally supposed that the virus reached its maximum concentration in the inoculated leaf within 7 to 10 days following inoculation. From the activity measurements, it was further found that the activity of the virus protein 5 days after inoculation was only about 25 per cent. of that of virus protein isolated 15 days later. Between the 5th and 10th days, the activity of this material almost doubled, and the increase continued until a maximum was reached about 20 days after inoculation. Therefore, not only the amount of virus protein but also the activity per unit weight of the material increased up to 20 days. This would indicate that virus in young lesions was lower in activity than that from somewhat older lesions. Subsequently, it was found that virus protein from the inoculated leaf was more active than that isolated at the same time from the top of the plant. This result might have been expected in view of the previous experiment, since it can be assumed that part of the virus lesions in the inoculated leaf were formed earlier than any of those in the top of the plant and therefore were somewhat older.

In regard to the characteristics of virus from young and old lesions, preliminary analyses with the ultracentrifuge have shown that a preparation from the old lesions had only one component. However, a sample of virus from young lesions prepared at the same time appeared to be made up of two components, one of which was about double the length of that of the other as determined by the sedimentation constant.

Experiments also indicate that nitrogen may be an important factor in increasing the activity per unit weight of virus *in vivo*. When nitrogen was withheld 10 days after inoculation, virus protein in the inoculated leaf continued to form at about the normal rate for a limited period, but the activity of this material, on a weight basis, remained fairly constant at the level reached about the time when nitrogen was last added. The activity at this point was about one half that ultimately displayed by virus protein from normal nitrogen-fed plants. Preliminary experiments have indicated that it may even be possible to increase this unit activity *in vitro* by supplying the virus with suitable forms of nitrogen.

The observations clearly show that virus in young lesions displays on a unit weight basis only a fraction of its potential biological activity and that such virus may vary somewhat in size and shape from virus isolated from older lesions.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

MEASUREMENT OF RESPIRATION AND GLYCOLYSIS OF A SINGLE SAMPLE OF TISSUE IN SERUM

WITH the development of a sensitive method of colorimetric analysis for lactic acid[1] and the "neutralized" serum technique for the manometric measurement of oxygen consumption of tissues suspended in serum,[2] it has become possible to determine in an ordinary Warburg vessel the rates of respiration and glycolysis[3] of a single sample of tissue suspended in serum. Respiration is determined manometrically and glycolysis chemically. At the beginning of the experimental period, a sample of approximately 0.2 cc of serum is tipped from the main vessel onto a few crystals of sodium fluoride in the side-well. The fluoride prevents glycolysis in the few cells which may enter with the serum. The tissue slices are prevented from being tipped in by first inclining the vessel in the opposite direction, allowing the slices to settle and decanting the serum. At the end of the experimental period, a 0.1 cc sample of this serum is analyzed for the initial lactic acid concentration. The serum remaining in the side-well is returned and mixed with the contents of the main vessel and a 0.1 cc sample of this serum is analyzed for the final lactic acid concentration. The amount of lactic acid produced by the tissue during the experimental period is given by the formula

$$X_{Lac} = (C - C_o)(V_o - V_1), \text{ where}$$

X_{Lac} = mg. of lactic acid formed during experimental period.
C = Lactic acid concentration in mg./cc. at end of experimental period.
C_o = Lactic acid concentration in mg./cc. at beginning of experimental period.
V_o = Volume of serum originally placed in vessel (usually 2 cc.).
V_1 = Volume of serum removed from side-well (usually 0.1 cc.).

[2] E. L. Spencer and W. C. Price, *Amer. Jour. Bot.*, 28: (in press), 1941.

[1] S. B. Barker and Wm. H. Summerson, *Jour. Biol. Chem.*, 138: 535, 1941.

[2] J. MacLeod and C. P. Rhoads, *Proc. Soc. Exper. Biol.*, 41: 268, 1939. A. Canzanelli and D. Rapport, *Amer. Jour. Physiol.*, 127: 296, 1939. C. O. Warren, *Amer. Jour. Physiol.*, 128: 455, 1940. D. G. Friend and A. B. Hastings, *Proc. Soc. Exper. Biol.*, 45: 137, 1940.

[3] Used in the restricted sense of meaning lactic acid formation.

During the experimental period, the oxygen consumption is determined manometrically in the usual way, with KOH in the center well to absorb CO_2. One advantage of this method over differential manometry for measuring tissue respiration in serum is that the time-course of respiration may be followed, and by using vessels equipped with more than one side-well, additional samples of serum may be withdrawn at intervals to follow the time-course of glycolysis.

Tests of the method show that the same figure for $Q_G^{O_2}$ is obtained by this "side-well technique," where only the serum is analyzed, as by the usual method of analyzing separate samples at the beginning and end of the experiment and including the tissue in the analysis. In the case of bone marrow, the only tissue so far studied by these methods, the Q_{O_2} is the same in neutralized as in unaltered serum,[4] but the $Q_G^{O_2}$ is higher in neutralized serum, apparently an effect of the absence of CO_2. This finding may not apply to other tissues.

These methods may of course be used with any medium, but they are particularly useful when it is important to use serum, to conserve tissue and to use as few manometers as possible. The chemical method of measuring glycolysis has the advantage of being virtually specific for lactic acid, and accuracy is gained by making the initial and final analyses on one sample of tissue rather than on separate samples, as in the usual way.

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THE PRESERVATION OF OXIDIZABLE SUBSTANCES IN SOLUTION[1]

CERTAIN chemical and biological preparations, notably those used for growth stimulation or containing natural sulphydryl complexes are prone to gradual oxidation and subsequent loss of potency when in solution. Shinohara[2] has given formulas for these inactivating oxidations and our own work Owen[3] on tissue extracts are in fair agreement with his findings.

After attempting to prevent oxidation in these solutions by refrigeration, tightly capping the vials and employing antioxidants with a minimum of success we found the following procedure to be effective. The embryonic extract or solutions are prepared in the usual manner either aseptically or with permissable amounts of preservative. These are then strained or filtered as desired. The preparation is then placed in open beakers or wide-mouthed screw cap vials and set at room temperature into a vacuum desiccator. The pump is started and sufficient vacuum applied to cause mild bubbling of the solution but little or no loss in volume by evaporation. Usually not over five minutes pumping is required to remove the dissolved air. The vacuum is then replaced with an inert gas, as carbon dioxide, slowly let in. The containers are filled to the top and the cap screwed on and tightened before removal from the desiccator. Low melting paraffin may also be used to seal the containers. All air must be excluded to insure protection from oxidation.

As an example 1:100,000 cysteine hydrochloride solutions and tissue extracts containing free sulphydryl were maintained at room temperature for over six months with no apparent loss of sulphydryl. Similar solutions not so treated usually gave a negative sulphydryl test after one to four weeks. The method of testing was that used by Owen[3] employing the phospho-18-tungstic acid reagent of Folin and Marenzi.[4]

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[4] C. O. Warren, *loc. cit.*

[5] This work was done during the tenure of a Lewis Cass Ledyard, Jr., Fellowship, New York Hospital, 1940–1941.

[1] Published with the permission of the Medical Director of the Veterans' Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the writers.

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