ON THE AMOUNT OF HEAT LIBERATED BY BACILLUS COLI WHEN GROWN IN THE PRESENCE OF FREE AMINO-ACIDS.

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In the course of some experiments on the estimation of the amount of heat liberated by B. coli when grown on various culture media, it was noticed that it produced little or almost no heat when grown on tryptic broth. This broth is prepared from casein by digestion with pancreatic extract and contains variable amounts (usually 2 p.c.) of a number of amino-acids. It was found that if the casein was well digested, the resulting fluid grew B. coli extremely well, and the heat liberated for a definite quantity of growth was always 6–8 times less than that produced from the same growth on ordinary nutrient glucose peptone broth.

It would seem that if the bacteria were supplied with sufficient free amino-acids ready to hand, surprisingly little or almost a negligible quantity of energy was lost in the process of their being built into the living substance. A comparison of broths digested for 1 week and those digested for a longer period (4–8 weeks) showed that the one week's digest always gave a much greater amount of heat for the same growth than that digested for 4 weeks. So within limits it seemed that the amount of heat liberated by B. coli when grown on tryptic broth was dependent on the length of time the broth had undergone digestion. The difference in this respect, however, seemed to be greater than could be explained by the further splitting of the polypeptids in the 4 weeks' fluid.

In other experiments evidence was obtained that when B. coli was grown under unfavourable circumstances, such as the absence of O or the presence of any cytolysing agent, the amount of heat liberated was greatly increased. In this respect resembling the Echinoderm egg, where Myerhof has shown (1) the amount of heat liberated in the unfertilised egg is greatly increased if the egg membrane has undergone cytolysis.

A great deal of variation was found in the amount of heat liberated by B. coli on different samples of tryptic digest. This was probably due to the fact that no attempt was made to standardise their content of amino-acids. The lowest amount of heat was obtained from one lot of broth that had been digested for an exceptionally long time (7–8 weeks) and had then been stored in the laboratory for almost a year. This

broth (about 5 litres) served for a large number of experiments and always gave the same result no matter how the experiments were varied. The results of the experiments described under 1 and 2 were obtained with it. None of the broths prepared subsequently gave such good results but their digestion was never carried so far or so thoroughly as in the first instance. The exclusion from these broths of any carbohydrates was confirmed by careful tests. The 1 week's digest was appreciably richer in tryptophane than the 4 weeks' digest, and its total solids somewhat greater, otherwise they seemed much the same.

In the present paper five typical experiments are described from among a large number carried out, which give a fair idea of the results obtained. It is hoped in future experiments where the amino-acid content of the broths will be standardised, more uniform figures will be attained.

Technique. The method used in making the heat estimations has been described by A. V. Hill(2). In the following experiments it has been modified in several minor points. It is based on the measurement of the relative temperature change taking place between the contents of two or more Dewar vacuum flasks, one of which contains water serving as a control, while the others contain the solutions whose heat production is to be measured. It has been shown by Hill, that slight alterations in the volume of the fluid contents of these flasks alters very considerably their rate of temperature fall. Thus by decreasing or increasing its contents, a flask can be made to have any desired rate of temperature fall. If an appropriate quantity of fluid is placed in each flask, any number of flasks can be given the same rate of temperature fall, that is the same rate of conduction of heat to the outside. They can then be employed in making a differential heat determination, provided they are uniformly exposed to the same external temperature variations. Thus errors due to external causes are almost entirely eliminated, and it is possible to carry out accurate heat determinations without being forced to use the complicated thermostats which are otherwise required. Hill has shown that it is possible to estimate to within 3 p.c. of its value, the liberation of 1 gram calorie per gram of flask contents in 10 hours, and in many of my own measurements I have been able to attain this degree of accuracy.

The ordinary narrow-necked silvered Dewar flasks were employed. These are made in two sizes as "refills" for commercial thermos bottles. The small size have a capacity of a little over 400 c.c., while the large hold slightly over 800 c.c. of fluid. On account of their greater volume, the larger flasks possess a coefficient of heat loss very considerably less

than that of the smaller, and for this reason are a little more accurate for making determinations, although their larger volume requires a much greater amount of culture fluid. I am indebted to Prof. Hill for placing at my disposal two 400 c.c. flasks, which were exceptional for having almost identical coefficients of heat loss. They could be employed differentially by placing the same amount of fluid in each. They will be referred to as flasks *R* and *L*. Their coefficient of heat loss when submerged in the water-bath at 37° C., was 0·0506. The greater part of this heat loss was due to conduction through the thick rubber stopper with which the flasks were closed when submerged in the bath. The large flasks used in these experiments (Exp. 3) and numbered 3, 4 and 5, when containing 790, 810 and 654 c.c. of fluid respectively, had almost identical coefficients of heat loss, *i.e.* $k = 0{\cdot}0403$.

The flasks were mounted in open wire-work baskets and arranged to clamp on the bath so that they were completely submerged in the water of the bath. Its temperature was kept at 37° C. by means of a thermo-regulator. The water in the thermostat was kept stirred by bubbling compressed air through it from a number of fine air-jets distributed evenly over the bottom of the bath. This stirring was sufficiently effective to render it impossible with a Beckmann thermometer to distinguish much more than a hundredth of a degree Centigrade between any two points in the water of the bath.

A copper constantan thermo-couple was connected to a sensitive Ayrton-Mather galvanometer with one junction in each flask, so any deflection of the mirror gave the difference of temperature between the two flasks. The sensitivity of the galvanometer was such that at a distance of 3·5 metres each millimetre on the scale represented ·00139° C. The flasks when submerged in the thermostat were closed by thick rubber stoppers. Through these, holes were drilled into which a short length of small glass tubing was inserted, which projected slightly above the upper surface of the cork, on this a small piece of rubber tubing was attached, which was long enough to reach well above the surface of the water when the stopper was in place in the flask, and the flask itself sunk down in the water of the bath, and down this tubing and through the cork the junction of the thermo-couple was inserted. These tubes served therefore the double purpose of introducing the thermo-couple junction, and at the same time of allowing any gases formed during the experiment to escape from the flask, without forcing out the cork.

The leads from the thermo-couples were brought to a specially constructed dial box furnished with a revolving key by means of which all

the thermo-couples could be put in circuit with the galvanometer in turn. It was arranged so that if this key pointed to the first figure on the dial, all the thermo-couples were short-circuited through the box, thus avoiding an injury to the galvanometer during the preliminary adjustment of the thermo-couples at the commencement of an experiment. A second key on the box, served to throw a number of resistances into the circuit at any time required. The wires of the leads, and all terminals, including those of the galvanometer, were made of copper throughout, thus eliminating any possible disturbing external thermo-electric effects.

The flasks after having their coefficients of heat loss determined in the manner described by Hill(1), were finally calibrated directly by means of the thermo-couples and the galvanometer. This method always gave a slightly smaller value for k, than that obtained by the use of the formula $\frac{T-T_0}{A-T_0}e^{-kt}$. In many instances, however, the two methods gave similar values for k. I have always preferred to use the figure given by the second method.

The procedure in carrying out one of the final calibrations was usually as follows. The proper amount of water was placed in a pair of flasks to give them similar values of k, this amount of water having been determined by the above-mentioned formula. One flask was then carefully adjusted with a Beckmann thermometer to have exactly the same temperature as the thermostat, which was 37° C., and trouble was taken to see that this control flask did not differ from the bath temperature by more than a hundredth of a degree C. The second flask (which was the one actually under calibration) was given a temperature half a degree C. above the first, both were then submerged in the water of the bath after having their stoppers and thermo-couple junctions placed in position. They were allowed to stand in the bath for an hour to warm up and were then raised till their necks were above the surface of the water, opened and their temperature readjusted finally. They were then resunk in the bath and after 30 or 40 minutes a series of readings were taken with the galvanometer extending over a period of three or four hours. These readings were then plotted out and k determined from the curve. The temperature of the thermostat was meanwhile carefully watched to see that it had not varied. To equalise the temperature throughout the contents of the flask it was gently shaken while in the bath a short time before each reading. The same method of stirring was also followed in making experiments as it was found impossible to stir the flask contents by the method recommended by Hill on account of the foaming

and frothing which takes place when air is bubbled through tryptic broth. Moreover the high temperature (37° C.) at which the experiments were carried out, made it impossible to use air on account of the evaporation and cooling which would have taken place.

To measure the actual heat production during any particular period of time, it is necessary to measure the increase of temperature of one flask over that of another at the end of the period, and multiply this by the capacity of the flask. In these experiments the capacity of the small flasks was 400 plus a correction of 20 for the material of the flask, giving a total of 420. In those experiments where the large flasks were employed the capacity was double this figure. If no heat were lost by conduction to the outside this product would represent the total heat liberated, but the Dewar flask in which the heat is being measured loses heat continuously, it is necessary that this heat loss be determined and added to the above value. The temperature fall during the period is the sum of all the falls of temperature at each instant. The rate of fall at any instant is measured by $k(T - T_1)$, where k is the coefficient of temperature loss and $(T - T_1)$ the difference of temperature between the two flasks at that instant. The total temperature fall is thus obtained by integrating $k(T - T_1)$ with respect to time, and this value is accurately obtained by measuring the area of a curve, plotting temperature differences (represented in galvanometer scale readings) against time.

Thus the total heat produced is equal to the capacity multiplied by the final temperature difference between the flasks plus this value just obtained, that is k (area of curve).

The heat production.

Exp. 1 (*a*). A quantity of emulsion of B. coli (24 hours culture) in saline solution (representing about ·1 of a gram of dried bacteria) was added to a mixture of peptone (1 p.c.) and glucose (about 4·5 p.c.). 2 grams of chalk were added, with a view to neutralising the acid formed during fermentation. After warming to 37° C. and thoroughly shaking, 400 c.c. of the mixture was introduced into flask *L*, and growth allowed to take place for 24 hours, the heat developed being measured at intervals. The exact quantity of the glucose introduced in 400 c.c. of fluid was found to be 16·8 grams. At the end of the experiment this had diminished to 11·6 grams. Thus 5·2 g. had been fermented. The heat produced during this period of time was 311·5 gram calories for the 400 c.c. of flask contents.

In this experiment about 1·6 p.c. of the energy contained in the

glucose was liberated as heat, but the whole of this heat was not produced from the glucose, for the living bacteria produce heat from the peptone solution in the absence of any sugar.

If we turn this heat value and growth weight into gram calories of heat produced per c.c. of flask contents we obtain the following value: The glucose peptone broth liberated in 22 hours, ·780 g. cals. per c.c. of flask contents.

Exp. 1 (*b*). A similar quantity (·1 gram) of the same culture of B. coli as that used in the first part of this experiment was added to 400 c.c. of Cole and Onslow's tryptic broth(3) and placed in flask *L*; flask *R* being used as a control with 400 c.c. water. The tryptic broth used was that mentioned on p. 50 as having been digested for an exceptionally long time. The stock broth had been diluted three times with an equal bulk of saline solution and its *p*.H adjusted to 7·4. The contents of the flasks were then warmed to the temperature of the water bath, the thermo-couples and corks placed in position and both flasks completely submerged in the water as described in the previous section.

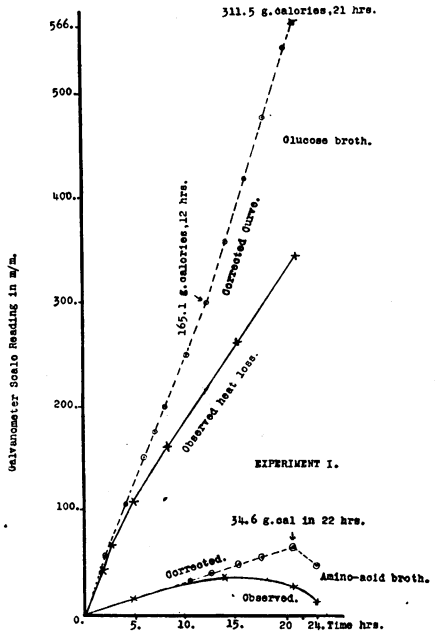

Fig. 1.

Fig. 1 shows the galvanometer scale readings over a period of 24 hours for this culture, and the curve corrected for heat loss shows that it gave off 34·6 gram calories of heat for the 400 c.c. of flask contents. The amount of growth that had taken place in the culture during this period, was determined by rough counts on the number of bacteria. The number in this culture was about the same as that of the previous glucose experiment. If anything there was a slightly greater growth in the amino-acid culture.

If we reduce the result of this experiment to values of gram calories per c.c. of flask contents, we obtain the following value: The amino-acid culture gave off in 22 hours ·085 g. cal. per c.c. of flask contents.

The previous glucose culture for the same amount of growth, gave

off, as said above, ·780 g. cal. per c.c. of flask contents, *i.e.* 8·5 times the amount of heat given off by the amino-acid culture.

In order however to compare with any degree of accuracy the magnitude of the process of growth with that of the resulting heat production, we need to know the weight of the bacteria concerned. In the subsequent experiments the bacteria were weighed after drying *in vacuo* at 37° C. This method has some drawbacks, but on the whole proved the most satisfactory. In some instances it was controlled by planting out the culture fluids by the three layer method and counting colonies.

Exp. 2 (*a*) (Fig. 2). To a solution of peptone and glucose (peptone

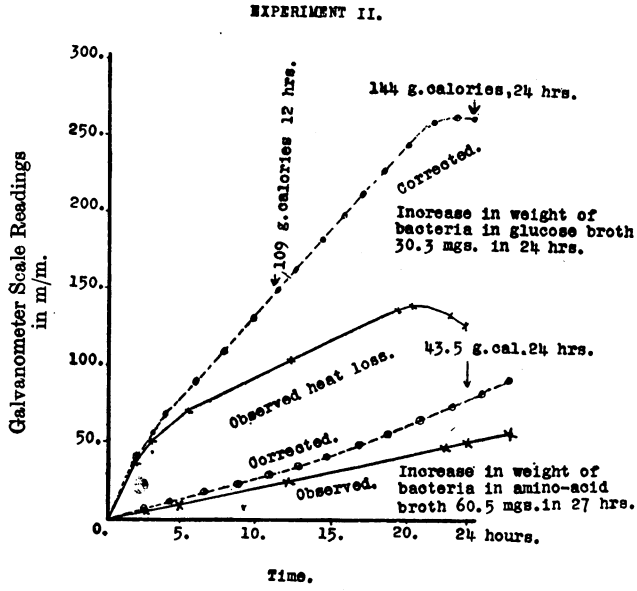

Fig. 2.

1 p.c. and glucose ·5 p.c.) was added 9·6 mg. bacteria (dried weight). The volume of the solution as before was 400 c.c., flask *L* being used for the culture fluid, while flask *R* was employed as the control with water. The experiment was continued for 24 hours, at the end of which time 144 g. cals. has been liberated. The weight of sugar which had disappeared from the solution as determined by reduction was ·99 or 1 gram approximately. While this experiment was in progress, the remainder of the broth used to inoculate the flask, was centrifuged and the weight of the bacteria in the deposit determined after drying *in vacuo* at 37° C. At the end of the experiment, the whole of the fermenting fluid in the Dewar

flask was likewise centrifuged, and the weight of bacteria determined again after drying. The weight of bacteria introduced at the beginning of the experiment was as already stated 9·6 mg. at the end of the experiment it was 40 mg. Thus considerable growth had taken place, and this had brought about the fermentation of fifty times its weight of glucose in 24 hours. If we turn these figures as before, into gram calories of heat produced per c.c. of flask contents per mg. of growth obtained in 24 hours, we have the glucose peptone broth producing ·0120 gram calorie per c.c. flask contents per mg. of growth.

Exp. 2 (*b*). One Roux bottle of a 13 hour growth on nutrient agar of B. coli was emulsified in 200 c.c. of amino-acid belonging to the same lot of digest as that used in the previous experiment. 25 c.c. of this emulsion was added to 375 c.c. similar broth contained in flask *L* and then was warmed to 37° C. The culture was maintained at this temperature in the thermostat as described in the previous experiments. The total heat produced in 24 hours was 43·5 gram calories. At the beginning of the experiment the weight of bacteria introduced into the flask was 9·5 mg., and at the end of the experiment this had increased after 27 hours to 70 mg. (in each case as before the numbers refer to material dried *in vacuo* at 37° C.). Thus in this culture 1 mg. growth gave ·0017 gram calorie in 24 hours[1] per c.c. of flask contents. In the previous portion of this experiment, the glucose gave ·0120 gram calories for the same amount of growth, thus the heat liberated by the glucose was seven times the amount produced by the amino-acid culture.

Exp. 3 (Fig. 3). In this experiment a fresh batch of tryptic broth was employed; this broth had only been digested a week and gave off a much larger amount of heat than that used in the two previous experiments. To 765 c.c. glucose (1·5 p.c.), peptone (1 p.c.) 25 c.c. of 24 hours culture of B. coli was added. This was shaken up and placed in flask No. 3 (large size). In flask No. 4, 810 c.c. water was placed as a control. In flask No. 5, 629 c.c. 1 week's digested tryptic broth was added. This broth had been diluted twice. To this flask 25 c.c. of the same emulsion of B. coli as that used for the glucose flask was added bringing its total contents to 654 c.c. Two thermo-couples were arranged to have one junction each in the control flask while the other junctions were in one case in the glucose culture and the other in the amino-acid flask. The contents of the flask were all warmed up to the temperature of the thermostat and the flasks sunk in the water of the bath. The experiment

[1] The weight was determined after 27 hours not 24, so the value given is not quite accurate; the error however is hardly enough to alter the value given above appreciably.

was continued for 24 hours and Fig. 3 shows the galvanometer scale readings with the corrected curve for heat loss over this period.

It was found that at the beginning of the experiment ·0154 mg. bacteria (dried) had been added to each flask. In the case of the glucose peptone culture, this had increased to ·0356 mg. and in this time the culture had given off 285 gram calories for 790 c.c. of flask contents. In the case of the tryptic digest this had produced in the same time ·0536 mg. growth, and given off 146 gram calories for 654 c.c. of flask contents.

If we reduce these values to gram calories per mg. of growth per c.c.

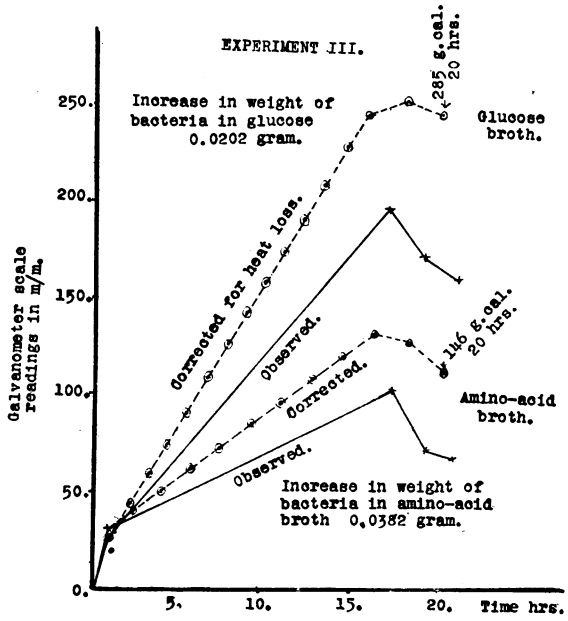

Fig. 3.

of flask contents as before, we find the glucose broth gave off ·0185 g. cal., while the amino-acid culture gave off ·0059 g. cal. per mg. growth per c.c. The glucose broth in this experiment gave off three times more heat than the tryptic digest for similar quantities of growth. Thus while in the first two experiments the tryptic broth gave off 7–8 times less heat than the glucose in this last, it only gave off three times less heat.

Exp. 4. In the experiment the amount of heat given off by the growth of B. coli on 1 week's tryptic digest was compared with that given off by the growth of the same organism on a 3 weeks' digest. Two litres of a 1 week's casein digest was divided in two equal portions, one

of which was immediately filtered, diluted three times with an equal bulk of saline solution, its p. H adjusted, sterilised and stored in the cold. The second portion, was digested for an additional 2 weeks, filtered, diluted in the same way, and its p. H adjusted like the first portion, to 7·4. The large flasks were used. An equal quantity of the same emulsion B. coli was added to both flasks (about ·015 mg.). The experiment was continued for 22 hours; in this time the weight of bacteria in the 1 week's digest had increased to ·2208 mg., while in the 3 weeks' digest it had increased to ·2720 mg., while the 1 week's digest gave off 704 g. cals., and the 3 weeks' digest 478 g. cals., for these amounts of growth respectively. In the case of the 1 week's digest, this works out at ·0054 g. cal. per mg. growth per c.c. flask contents in 22 hours, while in the 3 weeks' digest, this figure was ·0027 g. cal. per mg. per c.c. in the same time. Thus the amount of heat given off by B. coli when grown on 1 week's tryptic digest was almost twice that given off by the same organism on 3 weeks' digest, for the same amount of growth. This experiment was repeated three times with very similar results.

Exp. 5. In this experiment it was sought to determine the effect of the absence of oxygen on the amount of heat produced by B. coli when grown in glucose peptone broth. 400 c.c. glucose peptone broth (0·25 each) was placed in flask *R*, and a similar quantity in flask *L*. To each flask ·08 mg. B. coli was added. One flask was in free communication with the air, while the other (*R*) was rendered anaerobic by boiling the fluid under diminished pressure and replacing the air by nitrogen. The experiment was continued for 24 hours; at the end of this time about the same amount of glucose had disappeared from each culture, while the total dried weight of bacteria in each flask had not altered, planting out some of the fluid from each flask showed however that there were four times as many organisms alive in the aerobic culture than in the anaerobic, although this culture had given off 230 gram calories of heat in excess of the aerobic. The unhealthy bacteria of the anaerobic culture in spite of their fewer numbers, had given off considerably more heat than the healthy, in breaking down the same amount of glucose. Thus the normal process of metabolism would seem to be a far more economical one than the pathological.

A number of experiments were carried out in which various cytolysing agents were added to the culture fluids, such as bile salts, butyric acid, etc., growth of B. coli under these conditions almost always gave a greater amount of heat than under normal conditions for the same amount of growth.

Conclusions.

1. When B. coli is supplied with abundant free amino-acids already prepared, the process of building these into the living protoplasm is an extremely economical one, in which little energy is wasted. While different batches of tryptic digest gave widely divergent results in this respect (probably due to the fact that their amino-acid content was also very different), as compared with the growth of the same organism on glucose peptone broth, the process is from 3–8 times more effective, although in the fermentation of glucose this germ would seem to use surprisingly little (about 1 p.c.) of the total energy available in the carbohydrate molecule.

2. Tryptic broth that has been digested a short time (1 week) produces considerably more heat with B. coli than the same broth digested for a long time (3–8 weeks). This difference would seem to be far too great to be accounted for by the extra hydrolysis the polypeptids had undergone in the 3–8 weeks' fluid.

3. The pathological process of metabolism either with tryptic digest or any other culture fluid, is always accompanied by a greater liberation of heat than is given by the same amount of growth on the same medium under normal healthy conditions, so that pathological is less economical than normal growth.

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