for detecting microgram quantities of acid on the spot plate.

6. One hundred milligrams of Dee-O was dissolved in 10 ml of acetate buffer of *p*H 5.3 to 5.6, and about 1 g of scraped raw potato pulp was added. After it had stood 15 to 20 minutes, the reagent was filtered through glass wool. The raw potato is a convenient, essentially glucose-free source of peroxidase.
7. When *o*-tolidine dihydrochloride was used, 1 g was stirred with 0.45 g of KOH (85 percent pure) in 100 ml of 95-percent ethanol until it was dissolved, and the precipitated KCl was removed by filtration.

12 December 1956

Infrared Spectra of Mixtures of α- and β-D-Glucose Pentaacetate

Infrared spectra have been extensively used to identify sugars and their derivatives (*1*). The α- and β-anomers can also be differentiated through the presence or absence of certain characteristic peaks in their infrared spectra (*2*). In connection with some studies undertaken in this laboratory, it was proposed to use the intensity of an absorption peak characteristic of a particular anomer to determine the concentration of that anomer in solution.

In order to see whether or not this was feasible, we first investigated the infrared spectra of mixtures of the two anomers, α-D-glucose pentaacetate—mp. 112°C; $[\alpha]^{21}D$, +101° (EtOH, *c.*, 0.5) (*3*)—and β-D-glucose pentaacetate—mp, 133–134°C; $[\alpha]^{21}D$, +2° (EtOH, *c.*, 0.5) (*3, 4*)—in chloroform and acetone solutions. The α- and β-D-glucose pentaacetates were mixed in the proportions 1 α/4 β, 1 α/1 β, and 4 α/1 β at a total concentration of 1.00 percent (wt./vol.), and the infrared spectra were recorded with a Beckman IR3 recording spectrophotometer. This instrument permits one to obtain infrared spectra of compounds in solution without the spectrum including any contributions from the solvent.

Table 1 shows a series of absorption peaks found in the 8- to 15-μ region. The majority of those shown are apparently diagnostic of either the α- or β-anomer. The intensity of absorption is indicated by the figures in parentheses, which represent the percentage absorption, taking the absorption at 5.68 μ as 100 percent. The absorption at 5.68 μ was the same for both the anomers and their mixtures and was one of the most intense bands. There are also shown, for comparison, two peaks that are found in both α- and β-D-glucose pentaacetate. The α-anomer shows a slightly more intense absorption at 8.7 μ than the β-anomer. Both the anomers show essentially the same absorption at 11.15 μ.

Certain absorption peaks, such as that at 8.95 μ which is apparently characteristic of the β-anomer or that at 9.86 μ which is apparently characteristic of the α-anomer, decrease in intensity as the concentration of the anomer is decreased. On the other hand, the absorption peak at 9.65 μ which is characteristic of the β-anomer or the peak at 11.00 μ which is characteristic of the α-anomer rapidly disappears in the presence of the opposite anomer. It should be noted that the intensity of absorption is apparently not a factor. The absorption at 9.65 μ is very intense, while that at 11.00 μ is rather weak. Other examples will be apparent from Table 1. Essentially the same spectra were obtained when the solvent was allowed to evaporate and the solids run as films.

Since certain of the absorption peaks characteristic of an anomer behave as might be expected, and since the optical rotation of the mixtures was as calculated, we do not believe that there has been any interconversion between the α- and β-anomers. A possible explanation is that an association takes place in solution between the α- and β-anomers that suppresses the vibrations responsible for the infrared absorption characteristic of the anomer.

It would seem that considerable care should be taken when infrared analysis is used either for the identification or quantitative determination of sugars, particularly if there is a possibility that anomers are present (*5*).

F. A. H. Rice
L. C. Browning
Research and Development Department, U.S. Naval Powder Factory, Indian Head, Maryland

Table 1. Infrared absorption at several wavelengths of chloroform or acetone solutions of α- and β- and mixtures of α- and β-D-glucose pentaacetate. Total concentration, 1.00 percent (wt./vol.); wavelength in microns. The figures in parentheses represent the amount of absorption as compared with the absorption at 5.68 μ (percentage). The adsorption at 5.68 μ was the same for each anomer and for the mixtures.

β	4β/1α	1β/1α	1β/4α	α
8.70 (43)	8.65 (43)	8.65 (60)	8.65 (60)	8.65 (65)
8.95 (55)	8.95 (53)	8.95 (46)	8.90 (42)	
9.65 (92)	9.62 (94)			
	9.85 (50)	9.87 (73)	9.87 (73)	9.86 (78)
10.15 (26)	10.15 (26)	10.18 (25)	10.15 (25)	10.15 (24)
			10.35 (16)	10.35 (18)
10.43 (21)	10.45 (18)	10.45 (19)		
10.65 (62)	10.65 (49)	10.65 (53)	10.65 (23)	
10.75 (52)	10.75 (41)	10.75 (44)	10.80 (24)	
			11.00 (35)	11.00 (37)
13.45 (9)	13.45 (7)	13.45 (8)		
	14.05 (6)	14.05 (5)	14.15 (10)	14.17 (13)
14.45 (11)	14.45 (10)	14.42 (9)		

References and Notes

1. L. P. Kuhn, *Anal. Chem.* **22**, 276 (1950); E. S. Barr and C. H. Chrisman, Jr., *J. Chem Phys.* **8**, 51 (1940); H. G. Fletcher, Jr., and H. W. Diehl, *J. Am. Chem. Soc.* **74**, 5774 (1952); H. J. R. Stevenson and S. Levine, *Science* **116**, 705 (1952).
2. S. A. Barker *et al.*, *Chemistry and Industry* **1952**, 1156 (1952); S. A. Barker *et al.*, *J. Chem. Soc.* **1954**, 171 (1954); S. A. Barker *et al.*, *J. Chem. Soc.* **1954**, 3468 (1954).
3. C. S. Hudson and J. K. Dale, *J. Am. Chem. Soc.* **37**, 1264 (1915).
4. E. Erwig and W. Königs, *Ber. deut. chem. Ges.* **22**, 1464 (1889).
5. This report is published with the permission of the Bureau of Ordnance, Navy Department. The opinions and conclusions are those of the authors.

3 January 1957

Glycolysis by Tumor Mitochondria and the Action of Insulin

The cardinal role of glycolysis (formation of lactic acid from glucose) in the metabolism of living cancer cells has been recognized for well over 30 years (*1*). A high rate of glycolysis may also be readily demonstrated in cell-free homogenates prepared from tumors. However, the view is still widely held, despite evidence to the contrary (*2*), that the glycolytic enzymes of tumor cells (and of normal cells) are localized primarily in the nonparticulate fluid fraction of the cell cytoplasm. Mitochondria are regarded, at best, as potential stimulators of the glycolysis of the fluid fraction or else as playing some obscure part in the integrated functioning of the combined cell fractions. Mitochondria are generally considered not to possess the full complement of enzymes required to convert glucose into lactic acid.

During the course of investigations on hormonal regulation of the subcellular glycolysis of tumors, we have obtained new evidence of high intrinsic rates of anaerobic glycolysis by tumor mitochondria, provided that the mitochondria have been supplemented with supernatant fraction that has been enzymatically inactivated. Thus, when the supernatant fraction of a tumor homogenate

was boiled for 10 minutes, or pasteurized at 60°C for 10 minutes, and added to isolated mitochondria, lactic acid was formed from glucose at notable rates. Mitochondria not so supplemented showed only very low rates of anaerobic glycolysis. The supernatant fraction alone, after heat treatment, likewise produced negligible amounts of lactic acid from glucose as substrate. Illustrative data are given in Table 1.

The tumor mainly used in these experiments was the Cloudman S91 melanoma that was maintained by intramuscular transplantation in C × DBA/2 F_1 hybrid mice. Tumors were chilled rapidly after removal from the animals, and all subsequent operations prior to placing the manometric vessels on the Warburg bath were carried out in the cold. One volume of tissue was homogenized with 2 volumes of 0.25*M* sucrose in a glass tube fitted with a rotating plastic plunger. Homogenates were cleared of nuclei and cell debris by a 1-minute centrifugation with a maximum force of 8000g. The cleared homogenate was then further subjected to a 25- to 30-minute centrifugation at 20,000g. The supernatant fraction (containing microsomes) was thoroughly decanted, and the mitochondrial pellet was resuspended in sucrose solution to approximately one-third of the original volume of the cleared homogenate. Although the mitochondrial pellet was not washed, various lines of evidence indicate that the large activities observed in these experiments were not due to small amounts of adherent supernatant. For example, addition to the mitochondria of small quantities of unheated supernatant produced no such activities; and, as is indicated in a subsequent paragraph, qualitatively different responses to insulin were observed in glycolyzing mitochondria as compared with glycolyzing supernatant fraction.

In the presence of aliquots of either pasteurized or boiled supernatant, mitochondria glycolyzed, at 20°C, at nearly linear rates [$Q_{CO_2}{}^{N_2}(N) = 45$–70] for as long as 3 hours. Linear rates of glycolysis were also observed at temperatures as high as 40°C [$Q_{CO_2}{}^{N_2}(N) = 200$–$300$], although for shorter periods of time. Results similar to these with the S91 melanoma have also been obtained with mitochondria prepared from centrifuged Erlich ascites cells and Krebs-2 ascites carcinoma cells.

The heat-treated supernatant could be stored for many days at 6°C without loss of activity, but it was rendered inactive by dialysis. The activity of the heat-stable component was duplicated in small part by the following additions to and changes in the medium: adenosine monophosphate, 0.00017*M*; sodium pyruvate, 0.0068*M*; niacinamide, 0.02*M*; K_2HPO_4, 0.0075*M*; and $MgCl_2 \cdot 6H_2O$, 0.0062*M*. On the other hand, addition of a boiled extract of fermenting yeast cells resulted in mitochondrial activities approximating those obtained with boiled tumor supernatant (*2a*).

Experiments in progress indicate that the glycolysis by mitochondria is responsive to both insulin (see Table 2 for illustrative data) and anti-insulin hormones (for example, certain sex-hormone steroids), whereas this has not been found with glycolysis by unheated supernatant fractions prepared from the same tumors. Such a differential response constitutes a profoundly important distinction between the herewith reported mitochondrial glycolysis and the well-known glycolysis of the fluid supernatant fractions.

Recent research has demonstrated that the glycolysis and growth of certain tumors is under the control of a stress-modifiable, insulin-anti-insulin mechanism (*3, 4*), that operates at the hexokinase reaction (*5*). The data reported in this article (*6*) support the view that

Table 1. Anaerobic glycolysis by S91 mouse melanoma mitochondria. Each vessel contained 1.0 ml of a solution of the following components at the final concentrations given: 0.04*M* $KHCO_3$; 0.00098*M* $MgCl_2 \cdot 6H_2O$; 0.00075*M* K_2HPO_4; 0.00015*M* DPN; 0.00018*M* adenosine triphosphate (sodium salt); 0.01*M* glucose; and 0.25*M* sucrose. Mitochondrial suspensions were added in 0.3-ml aliquots (total nitrogen about 1.0 mg), and the fluid volume of each vessel was made to 2.0 ml with either the supernatant fraction treated in the manner indicated or with 0.25*M* sucrose. Lactic acid was determined by the Barker-Summerson method on the vessel contents at the conclusion of the experiment. The gas phase consisted of 95 percent N_2 and 5 percent CO_2 carefully freed of the usual traces of oxygen by passage over hot copper filings with small amounts of hydrogen. The temperature was 20°C. $Q_{CO_2}{}^{N_2}(N)$ = microliters of total acid produced (as indicated by the release of CO_2 from bicarbonate buffer) per milligram of total nitrogen of the mitochondria, per hour. $Q_{LA}{}^{N_2}(N)$ = microliters of lactic acid produced (as determined chemically) per milligram of total nitrogen of the mitochondria, per hour. Initial *p*H was 7.6.

System	$Q_{CO_2}{}^{N_2}(N)$				$Q_{LA}{}^{N_2}(N)$ (3 hr)
	1st hr	2nd hr	3rd hr	3-hr av.	
Mitochondria plus supernatant (60°C, 10 min)	57.1	55.4	53.8	55.4	51.5
Mitochondria plus supernatant (100°C, 10 min)	59.2	48.0	48.1	51.8	47.2
Mitochondria plus yeast extract (100°C, 10 min)	48.0	45.4	45.0	46.1	
Mitochondria	22.2	11.9	6.8	13.6	3.3
Supernatant (60°C, 10 min)	8.5	1.7	1.7	4.0	1.3

Table 2. Effect of insulin on anaerobic glycolysis by mitochondrial and supernatant fractions of S91 mouse melanoma. The system was the same as that described in Table 1 except that each vessel also contained 0.003*M* NH_4Cl. Subcellular fractions were prepared from the same tumors. The supernatant fraction was diluted 1/1 with 0.25*M* sucrose, and 1.0 ml (about 1.5 mg of total nitrogen) was added to each vessel. Low-zinc insulin (*8*) was dissolved in distilled water and added at the rate of 1 unit per vessel. $Q_{CO_2}{}^{N_2}(N)$ values are based on a 30-minute period. The rate of glycolysis by the supernatant fraction, prepared by the given procedure, was relatively constant from experiment to experiment with either glucose or glucose-6-phosphate as substrate. On the other hand, the rate of glycolysis by the mitochondria varied considerably with glucose but not with glucose-6-phosphate as substrate. Variable positive responses to insulin observed in a score of experiments are apparently related to the degree of glycolytic inhibition, at the hexokinase reaction, by endogenous antiinsulin hormones, especially as modified by host stress (*3*).

Experiment No.	$Q_{CO_2}{}^{N_2}(N)$ at 30°C					
	Mitochondria (plus heat-treated supernatant)			Supernatant		
	Minus insulin	Plus insulin	Percentage increase	Minus insulin	Plus insulin	Percentage increase
1	54.9	98.5	79.4	111.3	112.0	0
2	88.4	126.4	42.9	114.3	113.9	0
3	113.8	127.4	12.0	118.1	119.1	0
4	131.2	150.7	14.9	127.2	126.5	0
5	145.6	144.5	0			

mitochondria are the enzymatic sites of intense glycolytic activity and also the view (*3, 7*) that hormonal regulation of glucose phosphorylation in living cells takes place at the mitochondrial level of organization.

PAUL HOCHSTEIN*
Cytochemistry Section, Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland

References and Notes

1. O. Warburg, *Science* **123**, 309 (1956).
2. ———, *Metabolism of Tumors* (Constable, London, 1930), p. 110; H. G. Dubuy *et al.*, *Pigment Cell Growth* (Academic, New York, 1953); M. L. Hesselbach and H. G. DuBuy, *Proc. Soc. Exptl. Biol. Med.* **83**, 62 (1953); C. H. Gallagher *et al.*, *Biochem. J. (London)* 62, 436 (1956); H. G. DuBuy and M. L. Hesselbach, *J. Histochem. and Cytochem.* **4**, 363 (1956).

2a. *Note added in proof*: Since this article was submitted for publication, essentially the same results have been obtained in chemically defined media containing larger amounts of ATP. Details of these experiments are in preparation.

3. M. W. Woods *et al.*, *J. Natl. Cancer Inst.* **16**, 351 (1955).
4. J. C. Hunter, *ibid.* **16**, 405 (1955); M. W. Woods *et al.*, *Biochim. et Biophys. Acta* **12**, 329 (1953).
5. P. Hochstein *et al.*, *Federation Proc.* **14**, 227 (1955); S. P. Colowick *et al.*, *J. Biol. Chem.* **168**, 583 (1947); C. F. Cori, *The Harvey Lectures* (Science Press, Lancaster, Pa., 1946); V. S. Il'in and G. V. Titova, *Voprosy Med. Khimii* 2, 3, 203 (1956).
6. I wish to acknowledge the continued interest of Dean Burk, Mark Woods, George Hobby, and Jehu Hunter in these investigations.
7. O. Lindberg and L. Ernster, *Protoplasmatologia* 3(A4), 1 (1954).
8. Eli Lilly and Co., lot No. W-1302.

* U.S. Public Health Service postdoctoral research fellow of the National Cancer Institute.

3 January 1957

Analysis of Standard Granite and Standard Diabase for Trace Elements

The standard granite (sample G-1) and the standard diabase (sample W-1) have been used as interlaboratory calibration standards for analysis for major constituents of silicate rocks (*1*). In the course of this study, three sets of spectrochemical analyses of the trace elements were performed by R. L. Mitchell, K. J. Murata, and L. G. Gorfinkle and L. H. Ahrens. Partial trace-element analyses have been subsequently reported by other authors (*2*). The techniques used were neutron activation, stable-isotope dilution, and spectrochemical analysis.

Most of the trace-element data reported for standard granite and standard diabase have been obtained by direct-current-arc spectrochemical procedures. Although these techniques can give results reproducible to better than ± 5 percent, the accuracy is frequently off by as much as a factor of 2, because of large matrix effects, selective volatility of the elements, and so forth. The analyses reported here were performed by x-ray fluorescence techniques. The elements determined were copper, nickel, rubidium, zinc, strontium, zirconium, and manganese. The results are shown in Table 1, where they are recorded as parts per million (ppm).

X-ray fluorescence methods offer the advantages of excellent reproducibility and an exact method of accounting for matrix effects. The analyses were made with a North American Phillips x-ray fluorescence unit, using a tungsten target x-ray tube, a scintillation detector, and a helium atmosphere. Standards consisted of oxides of the elements in an aluminum oxide matrix. Selenium was used as the internal standard for elements below the absorption edge of iron (Zr, Sr, Rb, Zn, Cu, and Ni). The titanium already present in samples G-1 (1500 ppm) and W-1 (6530 ppm) was used as the internal standard for manganese.

The precision of the determinations of elements with concentrations higher than 25 ppm averaged ± 5 percent; the precision for elements with concentrations below 25 ppm averaged ± 10 percent. With the proper selection of an internal standard, the absorption corrections for the matrix should be accurate within a few percent. Therefore, the accuracy of the determinations reported here should lie within the combined errors of sample preparation, precision of counting, and the error of the matrix. The accuracy of the determinations for elements with concentrations greater than 25 ppm should be about ± 10 percent; for those below 25 ppm, about ± 15 percent. However, the accuracy of the Cu determinations is probably not this good because the CuKα radiation is partially interfered with by WLα_1 radiation originating from the x-ray tube, making a background correction difficult.

Since samples G-1 and W-1 are to be used by many laboratories as calibration samples for future analyses, every effort should be made to determine the trace elements in the best possible manner. Most trace-element analyses thus far have been performed by the frequently inaccurate spectrographic technique. This report is an attempt to contribute more accurate information on the trace elements in these important standards.

Table 1. Results of trace-element analysis of the standard granite (sample G-1) and standard diabase (sample W-1).

Element	Concentration (ppm)	
	G-1	W-1
Manganese	212	1400
Nickel	3	68
Copper	18	171
Zinc	26	78
Rubidium	248	25
Strontium	263	197
Zirconium	268	94

JOHN HOWER
THOMAS W. FANCHER
Research Center, Stanolind Oil and Gas Company, Tulsa, Oklahoma

References

1. H. W. Fairbairn *et al.*, *U.S. Geol. Survey Bull.* 980 (1951).
2. A. A. Smales, *Geochim. et Cosmochim. Acta* **8**, 300 (1955); L. F. Herzog and W. H. Pinson, *Geochim. et Cosmochim. Acta* **8**, 295 (1955); K. K. Turekian, *Trans. Amer. Geophys. Union* **36**, 532 (1955), abstr.

31 December 1956

Gravity Shields

In a recent paper D. C. Peaslee pointed out that so-called "antiparticles"—that is, particles that are related to normal particles by the *charge conjugation* operator of quantum theory—have positive mass and, hence, would be useless for employment in gravity shields (*1*). This argument is, of course, unexceptionable; however, it does not exclude the possibility of elementary particles with negative mass (whose antiparticles would possess negative mass as well), although such particles have not yet been discovered. There are some arguments tending to indicate that such particles may not be capable of existence at all (Pauli's derivation of the connection between spin and statistics), but they are not yet to be regarded as conclusive. In this contribution (*2*), I should like to describe briefly the behavior pattern of such particles if they should exist; I shall also demonstrate that such particles would be of very dubious value in "gravity shields."

For a particle of negative mass, according to Newton's second law ($f = ma$), force and acceleration point in opposite directions. Particles having masses of the same sign will attract each other gravitationally—that is, the *forces* will point toward each other—whereas particles of opposite masses will repel each other. Hence, all *accelerations* of particles of whatever mass will point toward particles of positive mass and away from particles of negative masses. If two particles having masses of opposite sign and equal magnitude act on each other purely gravitationally, then the particle of negative mass will chase the particle of positive mass, both their accelerations pointing in the same direction.

Let us now consider a particle of negative mass which also carries an electric charge. Under the influence of an applied electromagnetic field, such a particle will move according to the value of e/m—that is to say, a particle of negative