this is thalidomide and not a metabolite of thalidomide. The radioactive material in the urine is not thalidomide. Over a wide range (10-1000 mg/kg) an average of 30 to 40% of the radioactive label appeared in the urine in 48 hours. During this same period, most of the remaining radioactive label appeared in the feces.

The authors are indebted to Dr. A. Chisholm for suggestions concerning chromatographic solvent systems and to Drs. T. R. Blohm, W. L. Kuhn, and E. F. Van Maanen for their advice and interest.

1. Salter, C. E., Lodge-Patch, I. C., Hare, E. H., *J. Clin. Exp. Psychopath.*, 1959, v20, 243.
2. Jung, H., *Arzneimittel Forsch.*, 1956, v6, 430.
3. Somers, G. F., *Brit. J. Pharmacol.*, 1960, v15, 111.
4. Lasagna, L., *J. Chronic Dis.*, 1960, v11, 627.
5. Burley, D. M., *Med. World* (Lond.), 1960, v93, 26.
6. Beckmann, R., Kampf, H. H., *Arzneimittel Forsch.*, 1961, v11, 45.

Received September 13, 1961. P.S.E.B.M., 1962, v109.

Action of Antibodies on Metabolism of Normal and Tumor Cells *in vitro*. (27253)

F. FEO, P. DE GREGORIO AND LUDOVICA GABRIEL (Introduced by M. F. La Via)
Institute of General Pathology, University of Turin, Italy

Many workers have studied the cytotoxic action of antisera on normal and tumor cells *in vitro*. Some investigators used phase contrast microscopy or electron microscopy to study the morphological changes produced by the action of antisera(1-6), and changes in permeability of cytoplasmic membranes to dyes(7,8). Other experiments compared capacity of growth, *in vitro* or *in vivo*, after the action of antiserum(2,3,8,9,10).

In general these investigations showed that after a more or less prolonged incubation period in presence of antibodies, there were changes of the cytoplasmic membrane; swelling with formation of cytoplasmic blisters, with following swelling and fragmentation of the mitochondria; heaping of cytoplasmic granules around the nucleus, vacuolization and enhanced cell fragility, and, in the end, loss of cytoplasmic material. Ellem(11) showed an increased permeability of the cytoplasmic membrane to inorganic and organic acid-soluble phosphorus, which goes from the cell into the culture medium. Green *et al.* (12) observed that the incubation with antiserum elicits a decrease of RNA and cytoplasmic proteins, free amino acids, ribonucleotides and potassium, while DNA content remains unchanged. These changes are more evident in the presence of complement.

The action of antibodies on cell metabolism has been investigated less extensively. Flax (3) observed that under the action of immune gamma globulins, in presence of complement, Ehrlich ascites cells cannot utilize exogenous glucose by the oxidative pathway; on the contrary, they utilize succinate. Using the same cell type, Sugai and Aizawa(5) and Bickis *et al.*(6), showed that antiserum causes inhibition of the respiration in absence of substrates under the same conditions, anaerobic glycolysis is also inhibited, while the respiration with succinate is enhanced(6).

Recently it has been shown that normal human sera cause inhibition of aerobic and anaerobic glycolysis of sarcoma 37 ascites, and of anaerobic glycolysis of the Ehrlich and Krebs ascites carcinomas(8), as well as respiration of the latter 2 tumors(13). This action is complement-linked and may be due to presence of natural antibodies.

In our experiments we analyzed the behavior of respiration and aerobic glycolysis as a consequence of the action of specific antibodies, considering 2 cell lines; cells of normal kidney and of epidermoid carcinoma, cultured *in vitro*, and cells from the rat liver and Walker rat carcinosarcoma 256.

Methods and material. Normal kidney cells (PF) and epidermoid carcinoma cells of the floor of the mouth (KB) were cultured *in vitro* in Hanks' medium(14) supplemented

TABLE I. Effect of Various Serological Reagents on Oxygen Consumption of PF, KB, Walker Carcinosarcoma 256 and Rat Liver Cell Suspensions. Values are in micromoles per hr, per mg of nitrogen.

Additions	Cells			
	PF	KB	Tumor	Liver
Normal gamma globulin	1.43 ± .16	1.59 ± .06	.62 ± .06	.65 ± .07
Immune " "	1.66 ± .21	1.75 ± .06	.56 ± .12	.65 ± .06
Normal " " + complement	1.40 ± .20	2.25 ± .16	.39 ± .13	.54 ± .09
Immune " " " "	1.32 ± .08	1.75 ± .06	.46 ± .00	.57 ± .04

with additional 100 mg% of glucose. After 5 days in culture, the cells were removed from the culture flasks with 0.25% trypsin (Difco 1:250), washed in 0.15 M KCl and suspended in Ringer-Krebs phosphate medium at pH 7.4(15). The suspensions of rat liver cells and Walker carcinosarcoma were obtained by mincing the tissue and washing it several times with 0.15 M KCl. The final suspension was made in Ringer-Krebs phosphate medium.

The immune sera were prepared from rabbits by several inoculations of cell suspensions with adjuvant (1 ml of alumina gel plus 2 ml of a suspension containing 2×10^6 cells). Three intramuscular injections of 1 ml of suspension plus adjuvant were given over a 2-day period These were followed, after 3 days, by intravenous injection of 1 ml of adjuvant-free suspension. Ten days after the last injection the rabbits were bled by cardiac puncture and the serum kept at −20°C.

Normal and immune gamma globulins were prepared and partially purified from the serum of normal rabbits, or immune rabbits, by precipitation with Na_2SO_4(4). The titers of immune gamma globulins were tested by complement fixation. The reaction mixture contained 0.5 ml of each of the following: immune gamma globulins at the various dilutions; supernatant of homogenate of cell suspensions in 0.15 M NaCl, after centrifugation for 15 minutes at $3000 \times g$, containing 8 mg of protein/ml; guinea-pig serum (2 CU), anti-sheep serum (2 HeU), 2% sheep erythrocytes. Anti-Walker gamma globulins gave titers of 1/650 and 1/400 respectively against tumor and liver extracts. Anti-KB gamma globulins gave a 1/400 titer with extracts of KB and of PF cells, and anti-PF globulins a 1/200 titer with extracts of PF cells.

In some experiments with KB cells, immune sera were adsorbed at 37°C with KB cells before being used for preparation of globulins.

Oxygen consumption in the presence of glucose was determined with Warburg's direct method, in air at 37°C. Globulins (1.8 mg) were placed in the sidearm together with glucose (0.2 mM) and added to the cell suspension (1.5-2.0 mg of nitrogen) after 15 minutes of equilibrium. As a source of complement, 0.5 ml of normal rabbit serum at 1/10 dilution was used. The final volume was 3.5 ml. After incubating 60 minutes, the content of the flasks was pipetted into tubes containing 0.5 ml of 40% TCA. After centrifugation the lactic acid was determined on the supernatant according to Barker and Summerson(16).

Total nitrogen was determined according to Koch and Hanke(17), and proteins according to Bucher(18).

Results and discussion. Oxygen consumption in the presence of glucose by PF and KB cell suspensions undergoing the action of immune gamma globulins, with or without complement, did not exhibit any appreciable change as compared to controls. Anti-Walker gamma globulins also produced no change in oxygen consumption of Walker tumor and liver cells (Table I).

The slight decrease occurring in the respiration of KB cells exposed to immune gamma globulin and complement, was not significant, considering a confidence limit of the mean values for a student test of $t = 0.05\%$.

The difference between these findings and those of Flax(3) may be related to the fact that in Flax's experiments the metabolic change occurred when, very likely, the cells showed signs of suffering because of prolonged incubation.

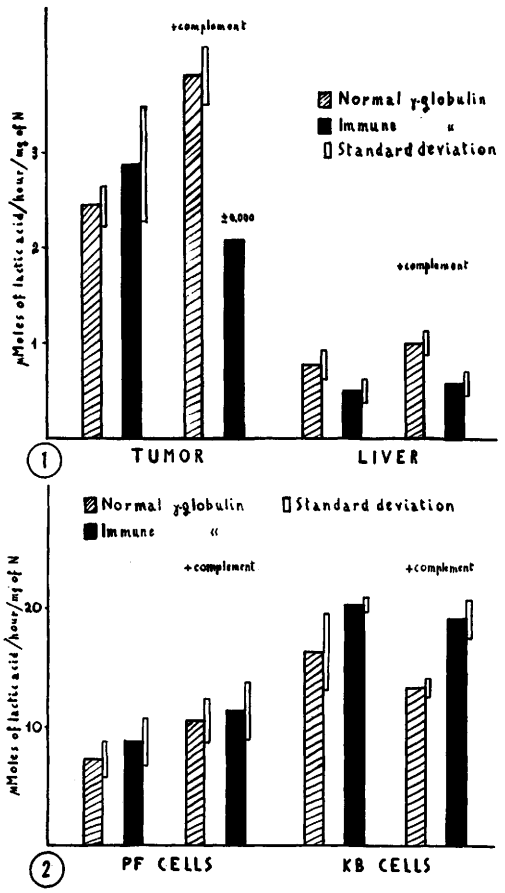

FIG. 1. Effect of various serological reagents on the aerobic glycolysis of Walker tumor and rat liver cell suspensions.

FIG. 2. Effect of various serological reagents on the aerobic glycolysis of PF and KB cell suspensions.

As to the action of anti-Walker gamma globulins on aerobic glycolysis of Walker tumor and liver cell suspensions, a decreased lactic acid production in the presence of complement was observed (Fig. 1). These results agree with the findings of Bickis *et al.* (6), Landy *et al.*(8), and Stambuk and Burk (13).

As to the study of aerobic glycolysis in PF and KB cells, while immune gamma globulins bring about no change in lactic acid production of PF cells, this production is appreciably enhanced in KB cells in presence of complement (Fig. 2). This enhancement does not occur if immune serum is adsorbed on KB cells before using them for preparation of immune globulins.

In some experiments PF cells were submitted to the action of anti-KB gamma globulins, but no change in respiration and aerobic glycolysis was observed, though anti-KB rabbit serum was found(9) to inhibit growth *in vitro* of both KB and PF cells.

This difference in behavior of both strains *in vitro,* as produced by the action of antibodies, and measured as respiration and glycolysis, is especially interesting since PF and KB cells, besides their morphological and cultural analogy, exhibit the same metabolic behavior(19,20).

As to the changes caused by antibody action in the metabolic behavior, measured as respiration and glycolysis, one might think that the adhesion of large globulin molecules first occurs at the cell surface, through which the respiratory exchanges occur. The immediate consequence of this adhesion may be damage to the mechanism of such exchanges.

It must also be pointed out that special enzymatic systems of various types of cells may exhibit a different degree of sensitivity to this deficit occurring in the respiratory exchanges, both in relation to the biological characters of the cell submitted to the action of the antibodies, and to quantity and quality of the latter.

On the other hand, it has not been proved that antibodies, as a consequence of the well-known changes occurring in cytoplasmic membranes, can penetrate into the cell and inhibit some enzymatic systems specifically. In any case, apart from a possible direct action of antibodies on enzymatic systems, the penetration of immune globulins into the cells, if occurring, certainly elicits changes in the colloidal state of the cytoplasm.

Furthermore, in agreement with Miller and Hsu(2), one would think that in this type of experiment there is on the one hand a heterogeneous antibody mixture, while on the other hand, these antibodies act on cells of a different age, some in mitosis, others with varying degrees of abnormality; *i.e.,* on cells which probably differ in sensitivity to the action of antibodies.

The cell suspensions used in these experiments, when examined by phase contrast microscopy during incubation with gamma glob-

ulins and complement, show a swelling after about 20 minutes affecting differently most, but not all, of the cells. This observation and the points mentioned above suggest that the action of antibodies is not always evident, in animal cells, as an "all or none" reaction.

The results presented here suggest that the metabolic changes observed after antibody action are not the consequence of a direct action on a given enzymatic system and that the study of glucose metabolism in cells submitted to antibody action is not always a useful indication of the cytotoxic effect of the latter.

Summary. The O_2 consumption of PF and KB cell suspensions cultivated *in vitro* and of Walker rat tumor and liver cells incubated with immune gamma globulins and complement, remains unchanged, while lactic acid production in aerobiosis by Walker tumor and liver cells, as a consequence of the action of anti-Walker gamma globulins and complement, shows a decrease. On the contrary, in the presence of specific antibodies and complement, an enhancement of aerobic glycolysis occurs in KB cells, while in PF cells no change is observed. It is suggested that some enzymatic systems may exhibit a different sensitivity to the altered metabolic exchanges occurring as a consequence of immune globulin adhesion to the cell surface.

1. Kalfayan, B., Kidd, J. D., *J. Exp. Med.*, 1953, v97, 145.

2. Miller, D. G., Hsu, T. C., *Cancer Res.*, 1956, v16, 306.

3. Flax, M. H., *ibid.*, 1956, v16, 774.

4. Goldberg, B., Green, H., *J. Exp. Med.* 1959, v109, 505.

5. Sugai, Y., Aizawa, K., *Nihon Univ. Med. J.*, 1959, v18/7, 1727.

6. Bickis, I. J., Quastel, J. H., Vas, S. I., *Cancer Res.*, 1959, v19, 602.

7. Defendi, V., Colter, G. S., *J. Nat. Cancer Inst.*, 1959, v23, 411.

8. Landy, M., Michael, J. G., Trapani, R. J., Achinstein, B., Woods, M. W., Shear M. J., *Cancer Res.*, 1960, v20, 1279.

9. De Gregorio, P., Terranova, T., Sabbatino, M., *Arch. Sci. Med.*, 1959, v108, 1.

10. McAllister, R. M., Grunmeier, P. W., Carriel, L. L., Marshak, R. R., *J. Nat. Cancer Inst.*, 1958, v21, 541.

11. Ellem, K. A. O., *Cancer Res.*, 1958, v18, 1179.

12. Green, H., Fleischer, R. A., Barrow, P., Goldberg, B., *J. Exp. Med.*, 1959, v109, 511.

13. Stambuk, B., Burk, D., *J. Nat. Cancer Inst.*, 1961, v26, 681.

14. Hanks, J. H., Wallace, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 196.

15. Umbreit, W. W., Burris, R. H., Stauffer, J. F., *Manometric Techniques and Tissue Metabolism*, Burgess Publ. Co., Minneapolis, 1951.

16. Barker, J. B., Summerson, W. H., *J. Biol. Chem.*, 1941, v138, 535.

17. Koch, F. C., Hanke, M. E., *Practical Methods in Biochemistry*, Williams & Wilkins Co., Baltimore, 1948.

18. Bucher, T., *Biochim. Biophys. Acta*, 1947, v1, 292.

19. Terranova, T., *Biochim. Appl.*, 1959, v6, 235.

20. Terranova, T., Feo, F., *Lo Sperimentale*, 1961, v111, 11.

Received September 19, 1961. P.S.E.B.M., 1962, v109.

Hypothalamic Temperatures and Blood Flow.* (27254)

ROBERT D. MCCOOK, CLARENCE N. PEISS AND WALTER C. RANDALL

Department of Physiology, Stritch School of Medicine and the Graduate School, Loyola University, Chicago, Ill.

It is generally assumed that the hypothalamus is warmed by the arterial blood which perfuses it(1,2). This belief is associated with the concept that changes in hypothalamic temperature, and resultant thermoregulatory discharges from it, are dependent upon changes in the temperature of the perfusing arterial blood supply. However, it is well known(3) that the metabolic heat production of a unit mass of gray matter in the central nervous system is greater than that of most

* Support for this research has come from the following sources: Chicago Heart Assn. and from Nat. Inst. of Health grant.