preparations in the animal body may be a factor in the results. It is also possible that results may be affected by contamination of the preparations with hormones other than ACTH. Pinto(9) reported that estrogen alone does not increase the weight of the adrenals of the hypophysectomized rat, but when administered along with ACTH induces an increase in adrenal weight greater than that produced by ACTH alone.

Brink *et al.*(10) have reported that ACTH peptides prepared by peptic digestion and tested both clinically in man and by the AAD assay in rats showed activity fully as great as unhydrolyzed ACTH concentrates. Our results, coupled with their observations, suggest that values obtained by the AAD assay will show closer correlation to clinical response induced than will values obtained by the AWI assay.

Summary. In the experiments presented, hydrolysis by peptic digestion or acid-heat treatment induced a marked loss of activity of ACTH as measured by adrenal weight increase, but these treatments had little or no influence upon the activity of the hormone as measured by adrenal ascorbic acid depletion.

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Effect of Cell Concentration on Oxygen Consumption of Leukocytes under Varying Conditions.* (19254)

JOHN D. HARTMAN.† (Introduced by N. L. Hoerr.)

From the Department of Anatomy, Western Reserve University, School of Medicine, Cleveland

Conflicting reports exist in the literature concerning the effect of cell concentration on the metabolism of leukocytes. Barron and Harrop(1) studied the metabolism of human leukocytes in autogenous plasma and found that neither glycolysis nor oxygen consumption increases in proportion to an increase in cell concentration. Soffer and Wintrobe(2) also found that oxygen consumption and leukocyte concentration do not increase proportionally. Ponder and MacLeod(3), however, reported that the oxygen consumption of rabbit polymorphonuclear leukocytes, suspended in buffered saline, is very nearly proportional to the number of cells.

The following work is offered as an attempt to reconcile these two seemingly opposing results and to clarify the extent of the disproportion existing between the cell concentration and the metabolism of leukocytes.

Materials and methods. Exudate leukocytes were collected from normal guinea pigs. Each guinea pig was injected intraperitoneally with 60 ml of a 1% sterile sodium chloride

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† Present address: Department of Anatomy, Temple Univ. School of Medicine, Philadelphia.

TABLE I. Mean Oxygen Uptake in Serum. Mean respiration rate and standard error of 10 experiments, calculated from first half hour O_2 uptake for each of 5 cell concentrations.

Cell conc., cells/mm³ × 1000	Mean O_2 uptake, 1st ½ hr		Mean respiration rate, mm³ O_2/million cells/ hr ± SE
	mm³	(Range)	
10.5	15	(11– 20)	.96 ± .06
21	18	(14– 21)	.59 ± .02
42	33	(29– 41)	.52 ± .02
84	57	(46– 76)	.46 ± .03
168	116	(99–141)	.47 ± .02

solution. After 16 hours the peritoneal exudate was withdrawn by gravity with a 16 gauge needle. The cell population at this time interval averaged 70 to 80% polymorphonuclear, and 30 to 20% mononuclear leukocytes. Eight to 10 guinea pigs were used for each experiment at a frequency no greater than once every 7 to 10 days. The pooled peritoneal exudate was centrifuged at 700 rpm for 2 minutes and the supernatant fluid decanted from the sedimented cells. The cells were suspended in 16 ml of a suspension medium by gentle pipetting. From this suspension 4 serial dilutions were made in such a way that the cell concentration of each succeeding dilution was half the cell concentration of the preceding dilution. Cell concentrations varying from 8000 cells/mm³ to 200000 cells/mm³ were used. Three suspension media were used: normal serum, peritoneal fluid, and a physiologic salt solution. Homologous serum was obtained from normal guinea pigs. The bicarbonate ion normally present in the buffer system of serum was reduced(4). The serum was then stored at —20°C until used. Peritoneal fluid was collected, as previously described, and all of the cells were removed by prolonged centrifugation. The bicarbonate ion in the supernatant fluid was reduced and the supernate stored at —20°C. The physiologic salt solution contained the following: NaCl 8.0 g, KCl 0.200 g, $CaCl_2 \cdot H_2O$ 0.147 g, $MgCl_2 \cdot 6H_2O$ 0.203 g, and distilled water to make 1000 ml. Immediately before suspending the cells, 1 part of a 0.5 M sodium phosphate buffer at pH 7.4 was added to 9 parts of the desired suspension medium. In those experiments dealing with the effect of pH, the desired pH of the suspension medium was obtained by varying the pH of the 0.5 M phosphate buffer. In those experiments testing the effect of phosphate, an organic buffer, tris (hydroxymethyl)-aminomethane (5), was substituted for the phosphate buffer. A concentration of 0.05 M tris in serum or peritoneal fluid gave a pH of 7.9.

The oxygen consumption of the leukocytes was measured on 3 ml duplicate samples by the direct method of Warburg. The cells were equilibrated at 37.5°C for 15 minutes and the oxygen consumption measured over a 2-hour period. Oxygen uptake measurements were made in an atmosphere either of air or of 100% oxygen. The reaction vessels were shaken at the rate of 120 per minute. The time from removing the cells from the animals until they reached the bath was 45 to 50 minutes. The pH of an aliquot of each cell suspension was taken at the beginning of the measurement of oxygen consumption and on the contents of each vessel after 2 hours in the bath. The respiration rates are expressed as mm³ oxygen/million cells/hour.

Results. When guinea pig leukocytes respire in buffered serum or buffered peritoneal fluid, the disproportion between cell concentration and oxygen uptake is apparent. In no instance from thirty experiments were the cell concentration and oxygen consumption proportional. The mean oxygen consumption of 10 experiments with the cells suspended in serum is shown in Table I.

Before the cell suspensions reached the constant temperature bath, the pH varied from 7.4 for the lowest cell concentration to 7.1 for the highest cell concentration. After the suspensions had been in the bath 2 hours, the pH varied from 7.4 for the lowest cell concentration to 6.8-6.9 for the highest cell concentration. The difference between any 2 successive cell concentrations seldom was over 0.2 of a pH unit.

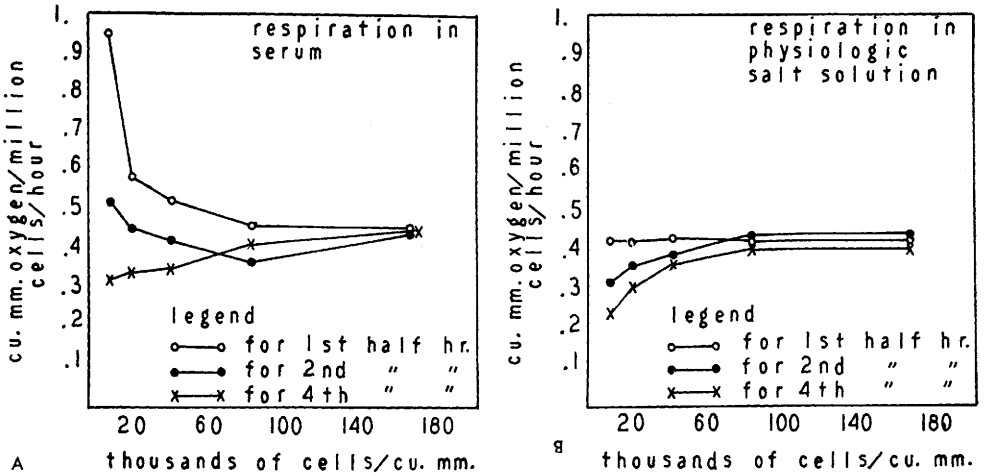

FIG. 1. Relationship of respiration rate of leukocytes to cell concentration at different time intervals. Respiration rates are calculated from the oxygen consumed during the first, the second, and the fourth half hour. Each point on the curves is the mean of 10 values. The curve for the third half hour is consistent with the above.

The effect of the experimental conditions on the disproportion between cell concentration and oxygen consumption was tested. To determine the effect of pH, the individual cell suspensions were buffered to give the lower cell concentrations lower pH values than the highest cell concentration, and so that after 2 hours in the bath the pH range between any 2 cell concentrations was not more than 0.2 of a unit (7.0-7.2). The effect of reducing the ionic strength of the suspension medium was tested at 0.02 M phosphate buffer concentration pH 7.4. The effect of excess phosphate ion was eliminated by buffering the cell suspensions with the tris buffer. To eliminate a possible limited diffusion of oxygen at the high cell concentrations, identical cell suspensions were run simultaneously in an atmosphere of air and 100% oxygen. At least 6 experiments were run at each of the above conditions. In no instance was there a change in the disproportion between oxygen uptake and concentration of the leukocytes.

Fig.1a and b show the difference between the respiration of leukocytes in buffered serum and in buffered physiologic salt solution. The difference between the means of the respiration rates at any 2 cell concentrations, during the indicated time periods, has been statistically evaluated by the t-test. The mean differences in respiration rate for the more critical comparisons are given in Table II. The results indicate that the disproportion between cell concentration and oxygen consumption, when leukocytes respire in buffered serum, is significant. The effect of time on the respiration rate, indicated by the change in shape of the curve at the different time intervals in Fig. 1a, is shown in Fig. 2. The differences in mean respiration rate occurring with time at each cell concentration have been statistically evaluated from data represented by the graphs in Fig. 1a and 2. The standard errors for such differences are of the same magnitude as those in Table II. These calculations indicate that for any one cell concentration, except the highest, the decrease in respiration rate with time is significant. From Fig. 2 it is apparent that the decrease in respiration rate with time becomes less as the cell concentration increases. Fig. 1a shows that with time the respiration rate at each of the 4 lower cell concentrations will pass from a phase wherein each is greater than that at the highest cell concentration, through a phase wherein each is equal to that at the highest cell concentration, to a phase wherein each is less than that at the highest cell concentration. In any one experiment the shape of the curve will depend upon the interval of oxygen consumption from which the respiration rates are calculated and probably upon the time inter-

TABLE II. Mean Difference in Respiration Rate Between Different Cell Concentrations Over 1st Half Hour and 4th Half Hour Oxygen Uptake. Mean difference and standard error calculated from 10 experiments with cells suspended in serum, and from 10 experiments with cells suspended in physiologic salt solution.

Between cell conc., cells/mm³ × 100	Mean difference in respiration rate, mm³ O_2/million cells/hr ± S.E. In serum		In physiologic salt sol.	
	1st ½ hr	4th ½ hr	1st ½ hr	4th ½ hr
105 & 210	.37 ±.05	.03*±.03	.01*±.03	.07*±.04
105 & 420	.44 ±.06	.02*±.03	.00*±.04	.13 ±.04
105 & 840	.50 ±.06	.09 ±.03	.01*±.04	.17 ±.04
105 & 1680	.44 ±.07	.14 ±.03	.01*±.04	.16 ±.03
210 & 420	.07 ±.03	.01*±.02	.01*±.02	.07 ±.03
210 & 840	.13 ±.03	.07 ±.02	.00*±.03	.10 ±.04
210 & 1680	.12 ±.02	.12 ±.02	.02*±.03	.10 ±.03
420 & 840	.06 ±.02	.07 ±.02	.01*±.01	.03*±.02
420 & 1680	.06 ±.02	.12 ±.02	.01*±.01	.03 ±.01
840 & 1680	.01*±.02	.05 ±.01	.02*±.01	.01*±.01

* Difference in respiration rate not significant.

val between removing the cells from the animal until they reach the bath.

During the first half hour, when leukocytes respire in buffered physiologic salt solution, the oxygen consumed is proportional to the cell concentration. This is indicated by the fact that the difference between the means of the respiration rates at any 2 cell concentrations over this time interval is not significant. However, with time the respiration rates of the 3 lowest cell concentrations decrease, so that after the first half hour the disproportion between cell concentration and oxygen consumption is significant.

Discussion. The results indicate that the conflicting reports in the literature are due to observations of leukocyte respiration under different conditions. The observations of oxygen uptake by leukocytes in serum confirm the reports of Barron and Harrop(1), and Soffer and Wintrobe(2). When the oxygen consumption of leukocytes is observed during the first half hour with the cells suspended in a physiologic salt solution, the results tend to agree with the finding of Ponder and MacLeod(3). That a disproportion becomes evident with time is not in agreement with Ponder and MacLeod, who state that the oxygen consumption of their rabbit leukocytes remained uniform over a prolonged period of time. Marinelarena(6), using guinea pig leukocytes which were collected essentially in the same manner as in this study but which were washed repeatedly, found that the oxygen uptake for such cells was uniform over a prolonged period of time. The leukocytes used in this study were not washed, since avoidance of all undue trauma to the cells was desired, as was avoidable delay in transferring the cells from the animals to the bath.

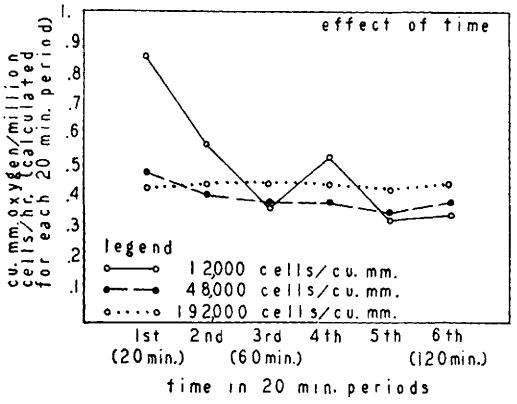

FIG. 2. Effect of time on respiration rates at different leukocyte concentrations with the cells respiring in buffered serum. Respiration rates (mm³ oxygen/million cells/hr) are calculated per 20 min period. Each point on the curve is the average of 6 experiments.

Summary. The conflict between reports in the literature, concerning the disproportion between cell concentration and metabolism of leukocytes, probably is due to observations made under different conditions. Measurements of oxygen uptake of leukocytes suspended in serum show the disproportion between cell concentration and oxygen consumption. When the oxygen consumption of unwashed leukocytes is measured in a physiologic salt solution, cell concentration and

oxygen uptake at first are proportional. However, with time there is a disproportional decrease in the oxygen consumed at the different cell concentrations.

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Use of Guinea Pig Eye in Study of Intraocular Infections Produced by Mumps Virus.* (19255)

VERN S. BOLIN AND GLEN R. LEYMASTER. (Introduced by Louis Goodman.)

From the Department of Public Health and Preventive Medicine and the Lockhart Memorial Research Laboratory, Pediatric Department, School of Medicine, University of Utah, Salt Lake City, Utah.

The injection of mumps virus into the guinea pig eye has been found to produce corneal opacity and result in the appearance of complement-fixing antibodies in the convalescent sera(1). The experiments in this paper deal with the use of the guinea pig eye in (a) the study of the neutralization of mumps virus by specific immune serum and pooled sera from normal mumps susceptible children. Further investigations were performed using the intraocular injection of mumps virus to demonstrate (b) the rise, persistence and decline of complement fixing antibodies in sera, (c) the persistence of mumps virus in ocular tissue, and (d) the corneal reaction and the subsequent appearance of complement fixing antibodies in the convalescent sera after the intraocular injection of 3 recently isolated strains of mumps virus.

Materials and methods. The mumps virus used in experiments I-III was Ender's embryo-adapted strain employed in the earlier study(1); it had undergone 54 serial passages in chick embryos. In the allantoic sac of chick embryos, the virus had a titre (ID_{50}) of $10^{8.3}$ 50% infective doses per ml. In the fourth experiment, 3 recently isolated strains of mumps virus were employed for intraocular inoculation. The JWE_2 virus was recovered from emulsified parotid tissue obtained from a child who had parotitis and meningoencephalitis at the time of his death(2), and had undergone 2 embryo passages. The $WTFE_6$ was recovered from aspirated testicular fluid obtained from a patient with orchitis, and the $135E_8$ from saliva obtained from a patient with parotitis(3). Each of these strains had undergone 6 and 8 embryo passages, respectively. The materials from which these 3 viruses were recovered were injected into the amniotic cavity of 7-day-old chick embryos. The titres of the viruses were not determined. The sera employed in the neutralization tests in guinea pig eyes were from 2 sources. The immune sera were obtained from the authors (V.S.B. and G.R.L.) 18 days after the intramuscular injection of 1 ml of mumps vaccine.† The complement fixing

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