oxazolidinedione (DMO) in skeletal muscle of dogs 1) infused with .3 N HCl, 2) infused with .3 N gluconic acid, 3) infused with HCl and hyperventilated to maintain extracellular fluid pH constant, 4) infused with .6 N HCO_3 and given CO_2 and O_2 to breathe to maintain extracellular pH constant, and 5) maintained in hemorrhagic shock. After the one to two hours required to reach equilibrium distribution of DMO there was not a striking difference in the intracellular hydrogen ion-extracellular hydrogen ion gradient between animals infused with HCl and those in hemorrhagic shock. Those infused with gluconic acid did show a significant difference from the shock animals. The differences were in the direction that would be expected from slower distribution across cell membranes of H^+ and/or HCO_3^- than CO_2. The experiments in which extracellular pH was maintained constant in the face of infusion of HCl or $NaHCO_3$ also demonstrate the primary dependence of intracellular pH on P_{CO_2} although in these experiments there is additional clear evidence of transfer of H^+ or HCO_3^- between intracellular and extracellular fluid over these short time intervals. With these time intervals of 1 to 3 hours, the intracellular acidosis of hemorrhagic shock is well reflected in the extracellular fluid, and the extracellular acidosis produced by infusion of acid into the blood is reflected in the intracellular fluid.

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Analysis of the Isoimmune Response to Leucocytes: I. Maternal Cytotoxic Response to Fetal Lymphocytes.* (32517)

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Considerable effort is being directed toward the antigenic analysis of human leucocytes and determining their role in histocompatibility(1). If it is established that these antigens are also determinants of histocompatibility, leucocyte typing ultimately will provide a simple means of matching donors and recipients for organ transplantation. A major source of antisera for these studies is found among women sensitized to antigens of fetal leucocytes during pregnancy. Although a number of studies concerned with the incidence of leucocyte isoantibodies among parous women have been carried out(2,3), in these studies leucoagglutination was exclusively used as an index of the antibody response. Our laboratory recently has had the opportunity to re-examine the immune response in parous women, by the now commonly used cytotoxic procedure(4), and to draw certain conclusions concerning the incidence and specificity of the antibodies manifest in this technique.

Materials and methods. Antisera. Blood samples were obtained from maternity patients during their hospital confinement at the time of delivery. The serum was separated and stored at −20°C.

Lymphocyte preparation. Our cell panel was obtained from 10 group O blood donors.

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TABLE I. Cytotoxic Screening of 1553 Maternal Sera Against a Panel of 10 Cells.

	Number of pregnancies									
	1st	2nd	3rd	4th	5th	6th	7th	8th-9th	10th & over	Totals
No. of positive sera	62	52	195	127	81	64	43	54	47	725
No. of sera tested	241	257	350	225	140	112	80	78	70	1553
% Positive	25.7	20.2	55.7	56.4	57.8	57.1	53.7	69.	67.	46.7

Lymphocytes were isolated by a gelatin sedimentation method. The blood was drawn, defibrinated, and immediately sedimented in freshly prepared 3% gelatin dissolved in .15 M NaCl. A 3:1 ratio of blood to gelatin was used. On incubation at 37°C, there was a separation of red cells and granulocytes from the lymphocytes. The lymphocyte-rich supernate was removed and centrifuged at a relatively slow speed to remove contaminating red cells. This supernate was then centrifuged at high speed to concentrate the lymphocytes. The cells were resuspended in Hanks' solution and counted. For storage in liquid nitrogen, the cells were suspended in an appropriate volume of Hanks' solution containing 10% AB serum and 10% DMSO, to give a cell concentration of 4 to 8 million cells/ml. Freezing of aliquots of cells was carried out at a controlled rate of 2°C/min to −40°C and 3°C/min to a temperature of −120°C. Cells were then stored in a liquid nitrogen specimen storage tank. When needed, the cells were thawed and washed in an equal mixture of Hanks' solution and normal saline.

Complement. Rabbit serum was used as the source of complement. Fresh rabbit serum was absorbed twice with washed packed human blood cells in a 20% concentration for 30 minutes at 4°C. The absorbed serum was titered for complement activity and tested for non-specific cytotoxicity.

Lymphocytotoxic test. The lymphocytotoxic test was performed in essentially the same manner as that described by Terasaki (4). Twenty-four antisera were plated per tissue culture dish (Falcon Plastics, Los Angeles, Calif.) in .001 ml amounts under mineral oil; this was done in advance and the plates stored at −20°C. Cells adjusted to 2,000,000/ml were mixed with rabbit complement in a ratio of 1 part cells to 3 parts complement; .006 ml of this mixture was added to each serum "dot." The test was incubated for 4 hours at 25°C with .002 ml formalin being added to each serum "dot" at the end of this period. Cytotoxic activity was determined from the microscopic cellular changes observed with an inverted phase contrast microscope (Unitron Instrument Co., Newton Highlands, Mass.). The viable lymphocyte has a non-granular, shiny appearance and the nucleus is indiscernible. In contrast, the injured lymphocyte is darkened and the nucleus easily recognizable. Through careful cell preparation and handling, the percentage of damaged lymphocytes in controls can be held to 1 to 3%. Cell controls consist of 3 or more dots to which Hanks' solution was added and an equal number to which a standard AB serum was added in place of antiserum. An assessment of cell viability was also possible among the test antisera, since the vast majority of antisera being tested with a given cell were negative or approached control values. With practice, the percentage of cells having undergone cytotoxic changes could be reasonably ascertained from a simple scanning of a number of fields. For screening purposes, only those sera producing cytotoxic changes in 50% or more of the cells were considered to be positive.

Results. Cytotoxic screening. A total of 1553 maternal sera were screened against a panel of lymphocytes obtained from 10 individuals. As shown in Table I, 46.7% of the sera tested were positive with one or more panel cells. After a single pregnancy, positive reactions were obtained with 25% of the sera. After 3 pregnancies, the percentage of positive sera rose to 55% and remained at this level through 7 pregnancies. The sera from 148 women having had 8 or more pregnancies showed positive reactions in 68%.

Reactions of individual screening cells.

TABLE II. Percentage of Positive Reactions Obtained With Each of the Panel Cells.

	Cell No.										
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	Total
Positive reactions	171	221	133	215	204	347	388	284	234	238	2435
% Positive	7.0	9.1	5.5	8.8	8.4	14.3	16.0	11.7	9.6	9.8	

The contribution of each of the screening cells to the number of positive reactions is shown in Table II. Of the total of 2435 positive reactions recorded in this study, the maximum number (16%) was contributed by cell C-7, and the minimum number (5.5%) by cell C-3. These differences, observed in the reactivity of the screening cells, caused us to consider what our results might have been if fewer and/or less reactive panel cells had been employed for screening. Accordingly, the cells were arranged in the order of reactivity shown in Table II. The number of sera giving a positive reaction with the least reactive cell in this sequence (C-3) was determined; the number of additional sera giving a positive reaction with the next least reactive cell was then determined, etc. In this manner, the contribution of each cell to the progressive increase in the percentage of positive sera being detected could be determined. The results presented in Fig. 1-A show a comparatively constant percentage increase with each additional cell. It may be noted that 78% of the positive sera would be detectable after testing with the 8 least reactive cells. This data may be contrasted to that shown in Fig. 1-B, in which the cells are arranged in a decreasing order of reactivity, *i.e.*, the reverse of that shown in Fig. 1-A. In this order, 78% of the positive sera would be detectable after testing with only the first two most reactive cells.

Specificity and number of pregnancies. Specificity can be estimated from our data only in terms of the number of cells with which an antiserum is reactive, *i.e.*, an antiserum which is reactive with fewer cells is more likely to be made up of antibodies specific for fewer antigens than an antiserum reactive with many cells. In Table III the percentage of positive sera reactive with different numbers of cells is shown. A major indication of specificity is that an average of 35.5% of all positive sera were reactive with only one panel cell. A maximum value of 50% was obtained for antisera from first pregnancies and a minimum value of 23.6% for antisera from second pregnancies. Further, it may be noted that 80% of all positive sera were reactive with 4 or fewer panel cells, and that the percentage of anti-

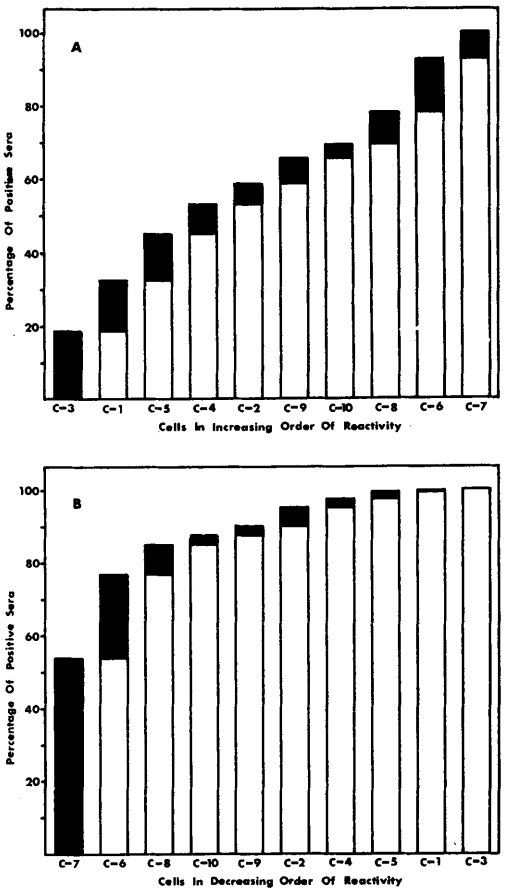


FIG. 1.-A and 1-B. Progressive contribution of each member of the cell panel to total number of positive sera. In Fig. 1-A cells are arranged in *increasing* order of reactivity. In Fig. 1-B cells are arranged in *decreasing* order of reactivity. The increase in percentage of positive sera detected with each additional cell is indicated by black portion of each column.

TABLE III. Comparison of Antisera, Obtained After Increasing Numbers of Pregnancies, as to the Number of Cells with Which Each Antiserum Is Reactive.

No. of pregnancies	No. of cells reactive with an antiserum									
	1 cell	2 cells	3 cells	4 cells	5 cells	6 cells	7 cells	8 cells	9 cells	10 cells
1st	50.0*	14.0	10.9	7.8	7.8	3.2	4.7	1.6	0	0
2nd	23.6	18.2	14.5	7.3	12.7	5.5	9.1	7.3	0	1.8
3rd	33.0	16.8	14.7	8.6	8.6	8.1	2.5	3.1	3.1	1.5
4th	28.6	15.1	12.7	11.9	7.9	5.5	7.1	5.6	4.0	1.6
5th	38.3	24.7	7.4	4.9	4.9	2.5	2.5	6.2	4.9	3.7
6th	34.4	10.9	6.2	12.5	7.8	7.8	7.8	6.3	4.7	1.6
7th	34.1	20.5	6.8	13.6	6.8	2.3	9.1	4.5	0	2.3
8th & 9th	40.0	5.5	12.7	3.6	10.9	7.3	9.1	5.5	5.5	1.8
10 or more	37.5	14.6	12.5	8.3	6.2	4.2	6.3	8.3	0	2.1
Avg	35.5	15.6	10.9	8.7	8.2	5.2	6.5	5.4	2.5	1.8

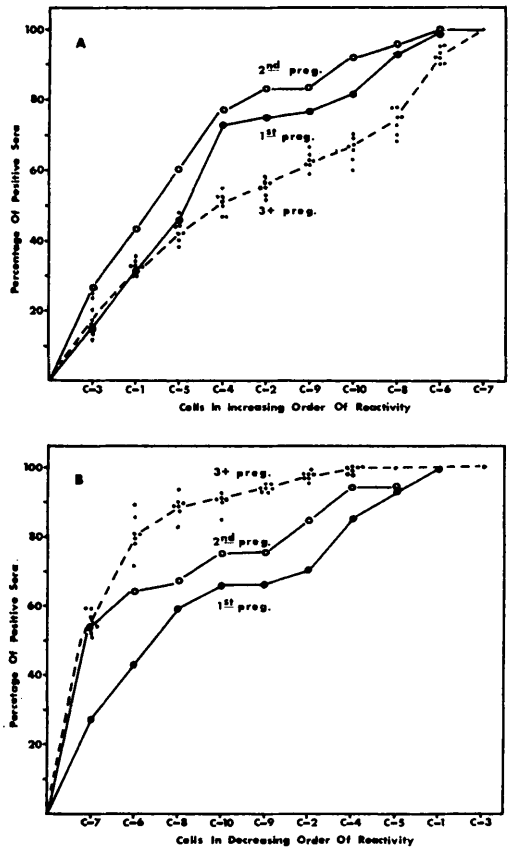
* Percentage of positive sera.

sera reacting with a greater number of cells did not increase with the number of pregnancies.

To carry the point of specificity a step further, the reactivity of the antisera for individual panel cells was considered. In Fig. 2-A and 2-B, the cells were again placed in the order of their reactivity, *i.e.*, in an increasing order of reactivity in Fig. 2-A, and in a decreasing order in Fig. 2-B. It can be seen that the antisera from women having had 3 or more pregnancies show similar reactivity for the various panel cells. In contrast, the antisera from both first and second pregnancies show a divergent pattern of reactivity. This distinguishing expression of specificity found with antisera from first and second pregnancies will be considered in the discussion.

Discussion. Analysis of our data (Table I) as to the effect of the number of pregnancies on the incidence of cytotoxic antibodies showed that cytotoxic antibodies were found in 25% of women after a single pregnancy. The incidence decreased to 20% with the second pregnancy and then rose sharply on the third pregnancy to 55%, to remain at this level through 7 pregnancies. Above seven pregnancies, an incidence of 68% was found. An overall incidence of lymphocytotoxic antibodies among parous women was found to be 47%. These values are much higher than have been reported previously for leucoagglutinins(2), *i.e.*, 18% is commonly considered to be a representative frequency of leucocyte isoantibody formation among women having had 3 or more pregnancies.

As shown in Table II, the 10 screening cells employed in this study varied with re-



FIGS. 2-A and 2-B. Progressive contribution of each member of the cell panel to total number of positive sera obtained after different numbers of pregnancies. Fig. 2-A cells are arranged in *increasing* order of reactivity; Fig. 2-B cells are arranged in *decreasing* order of reactivity. Only a single curve is shown for values obtained with antisera from women having had 3 or more pregnancies.

gard to their capacity to react with the various antisera; cell C-3 accounted for a minimum of 5.5% of the positive reactions and cell C-7 accounted for a maximum of 16% of the positive reactions. The extent to which these differences were due to the presence of the same antigen on more than one panel cell is illustrated in Fig. 1-B. In this Figure, it is shown that 78% of all the positive sera would have been detected employing only the 2 most reactive cells of the panel, indicating that the majority of lymphocyte isoantigens were represented on these 2 cells. This finding suggests that antibody screenings could be performed with cells from a few select individuals. Such a selection would provide a simplified screening panel, similar to that routinely used for detecting antibodies to human red cell isoantigens(5).

Utilizing the criteria that antisera reacting with fewer cells are correspondingly more specific than antisera reacting with many cells provided a means whereby our data could be analyzed for specificity.† On this basis, a high degree of specificity was demonstrated among the maternal antisera in that 35.5% of all the sera tested were reactive with only a single cell (Table III). Further, the degree of specificity did not appear to be less with increasing numbers of pregnancies, as attested by the fact that the proportion of antisera reactive with a greater number of cells did not increase with the number of pregnancies.

Several peculiarities of the lymphocytotoxic response noted in first and second pregnancies may be explained on the basis of a previous observation made concerning human A isohemolysins(6). The injection of hog A substance into B or O individuals resulted in a rise of anti-A hemolytic activity, which was characteristically followed by a decline that could not be impeded by repeated antigenic stimulation. Cytotoxic antibodies to *specific* lymphocyte isoantigens would appear to have followed a similar course. It is correspondingly judged that specific cytotoxins were frequently formed during a first pregnancy to one or more lymphocyte isoantigens. These antibodies declined in concentration during the second pregnancy and essentially disappeared by the third pregnancy. Such a response pattern to specific isoantigens could readily account for: 1) the difference in specificity manifest by antisera from first and second pregnancies (Fig. 2-A and 2-B); 2) the high proportion of antisera (50%) from first pregnancies found to be specific for a single member of the cell panel (Table III) and 3) the incongruous, low incidence of cytotoxic antibodies found among women with second pregnancies (Table I).

Summary. The screening of maternal sera for lymphocytotoxic antibodies against a panel of 10 cells showed a 46.7% overall incidence of antibody formation. Positive reactions were obtained with 25% of the antisera obtained from women after a single pregnancy and 68% with antisera from women having had 8 or more pregnancies. As judged by the number of panel cells with which an antiserum reacts, there was no discernible tendency toward decreasing specificity with increasing numbers of pregnancies. It was observed, however, that antisera from first and second pregnancies differed in specificity from those antisera obtained after 3 or more pregnancies. A basis for this, and related observations, is discussed.

We would like to thank Dr. A. Lash of the Cook County Obstetrics and Gynecology Department for allowing us to obtain maternal blood samples. We also would like to acknowledge the technical assistance of Mrs. M. L. Catino and Miss H. Halicka.

† This criteria obviously ignores the frequency with which a given antigen is present in a population. As a result, an antiserum specific for a single antigen which is present in a high proportion of the population would not be classified as being specific in our analysis. However, this point should not appreciably alter the validity of our conclusions, since antisera reactive with the majority of the panel cells were rare.

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Relationship of Diet Composition to Survival Time of Chicks When Subjected to High Temperature.* (32518)

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Several factors influence survival time of poultry during heat stress. It has been reported that heat tolerance is controlled by inheritance(1-3), and that genetic selection is effective in decreasing or increasing the survival times of birds(2). Laying hens developed in an area of high temperatures were more tolerant of high temperatures than those developed in cooler areas and those developed in cooler areas were more tolerant of cool weather(3). It has also been found that temperature and relative humidity affect the heat tolerance ability of poultry(4).

There are relatively few reports in regard to the effect of nutrition on heat tolerance. Most of the research has been concerned with increasing the feed efficiency, egg production and/or growth during the summer months. Elevated temperature depressed growth and feed consumption, and the incorporation of fat into the diet improved growth and feed consumption(5). Mortality was shown to be appreciably greater in groups of chickens receiving diets moderately high in protein than it was in groups receiving standard diets or diets low in protein(6).

It is an accepted fact that the chicken eats to meet its energy requirement. There has been, however, much controversy as to whether calories from fat, carbohydrate and protein are equal. It has been reported that calories from fat and carbohydrates are equal (7). When protein, fat or carbohydrate was fed alone, the basal metabolism was raised over the intake of calories received from the individual feedstuffs, with protein giving the greatest increase(8). All specific dynamic effects were reported(9) to be greatly modified when a food mixture rather than a single foodstuff was fed. It has also been reported (10) that the iso-caloric substitution of corn oil for 0, 5, 10 and 15% glucose and animal fat resulted in a reduced heat increment at 90°F as the level of corn oil was increased. However, increasing the energy or protein content of the diet did not restore growth rate of chicks when the temperature was increased from 70 to 95°F(11).

This study was designed to determine the relationship of dietary protein and energy levels to the heat tolerance of five-week old Single Comb White Leghorn chicks.

Methods. Experiment 1. Birds used in Trials 1 and 2 were the F_3 generation of the lines selected for high and low tolerance to high temperature(2). These birds were designated as hi-line and lo-line birds, respectively.

At one day of age the chicks were wing-banded for identification and equal numbers of hi-line and lo-line chicks were randomized into floor brooding pens with 20 chicks per pen. The chicks were not dubbed, debeaked, wingclipped or vaccinated. Each pen contained peanut hulls as litter, one automatic water fount, one tube-type feeder and one infra-red bulb as a heat source.

The diets (Table I) used were corn-soybean type containing 2026 Calories of productive energy per kilogram of diet. Adjustments were made by varying the corn, soybean meal and inert filler (sand) to keep the diets isonitrogeneous and iso-caloric as animal fat was added. Feed and water were available *ad libitum*.

Survival time, at 40.8°C, of the 5-week old chicks was measured in a heat chamber as described by Wilson *et al*(2).

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