sent the error inherent in the procedure. Since the variations in the experimental tubes were much greater than in the control tubes, and were always in the same direction, the results suggest that the ascorbic acid in the liver homogenate was bound to some other substance, forming a complex that gives rise to a density gradient under ultracentrifugation. The data are considered evidence favoring the assumption that ascorbic acid exists in tissues partly in a bound form. The pellets of mitochondria and microsomes removed after the second and third contrifugations were analyzed for ascorbic acid. They contained 1 to 5% of the total ascorbic acid, values that must be considered high as the particles were not washed.

Summary. 1. The procedure of Sumerwell and Sealock, which yields evidence in favor of the exsitence of ascorbic acid in liver tissue in a bound form, was repeated. Results were in essential agreement with the work of these authors. 2. A study of the behavior of ascorbic acid in an aqueous liver homogenate under ultracentrifugation was made. At a centrifugal force of 100,000 $\times$ g a significant density gradient of ascorbic acid was formed. 3. Addition of urea in 3 N and 6 N concentrations had no influence upon the density gradients of ascorbic acid formed in aqueous liver extracts submitted to high speed centrifugation. 4. The results obtained are interpreted as evidence in favor of the assumption that ascorbic acid exists in tissues in a bound form.

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Warburg Studies on Coccidioidin. (28453)

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In most studies of the mechanism of increased oxygen uptake associated with phagocytosis peritoneal exudate cells from guinea pigs and rabbits have been used(1-3).

Strauss and Stetson's Warburg studies on the effect of *in vitro* addition of macromolecular substances to lightly heparinized human blood have indicated a stimulation of oxygen uptake upon addition of bacterial endotoxins and soluble antigen-antibody complexes(4). The effect has been attributed to stimulation of respiratory activity of blood leucocytes.

In vitro addition of substances to whole blood samples provides an approach using leucocytes of peripheral blood under more physiological conditions than usual. Since skin sensitivity to coccidioidin has been passively transferred with leucocytes from sensitive donors(5) it seemed worthwhile to investigate *in vitro* addition of coccidioidin to whole blood samples from coccidioidin negative and positive skin reactors.

Materials and methods. Blood from apparently healthy individuals was drawn from the antecubital vein into a heparinized syringe so that the final concentration of heparin was 3 units (30 μg) per ml of whole blood.

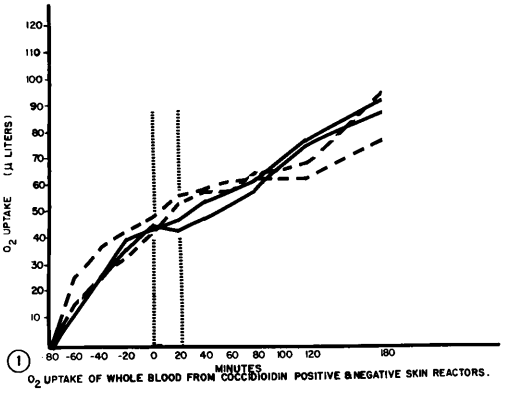
Smith's lot 64D4 skin test antigen received from the San Fernando VAH was used throughout this study. Skin tests were performed by intradermal injection of 0.1 ml into the left forearm using a 1:1000 dilution. Those individuals with a negative reaction were tested similarly with a 1:100 dilution. Readings were taken at 48 hours.

Manometric determinations. Consumption of O_2 was measured by the direct method of Warburg. Upon withdrawal of blood by venipuncture, the blood was mixed by gently inverting the syringe several times. Aliquots of 2.8 ml were then measured directly into the reaction chambers of duplicate 15 ml single side arm Warburg flasks. The center well of each flask contained 0.2 ml of 20%

KOH and the side arm 0.2 ml of 1:100 Lot 64D4 coccidioidin. After a period of equilibration at 36°C readings were taken at 4—20 minute intervals and 6—20 minute intervals after tipping in the coccidioidin.

Smears. Duplicate conventional blood smears were made before and from the Warburg flasks upon completion of the manometric measurements. One set was stained by Wright's method and examined for evidence of leucocyte degeneration. The second set was stained with Acridine Orange(6) and examined by fluorescent microscopy for attachment of coccidioidin.

Results and discussion. Fig. 1 indicates typical individual curves of 2 positive and 2 negative skin reactors. Oxygen uptake in the first 20 minute period after addition of the coccidioidin is increased in the positives while it is decreased or remains the same in the negatives.



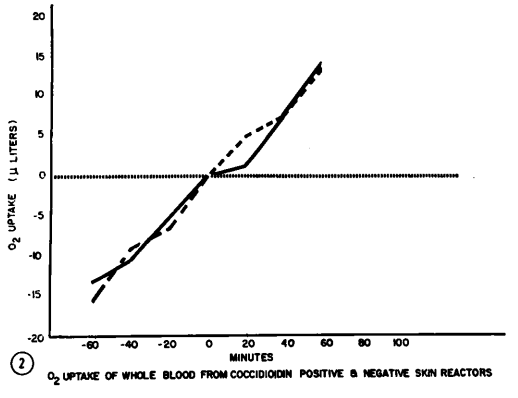


FIG. 1. Individual blood sample curves. Each point is avg of duplicate samples. Dotted line = positive skin reactor blood. Solid line = negative skin reactor blood. Coccidioidin added at 0.

FIG. 2. Avg curves. Duplicate blood samples from 11 positive and 8 negative coccidioidin skin reactors. Dotted line = positive skin reactor blood. Solid line = negative skin reactor blood.

TABLE I. Warburg Results of Coccidioidin Addition to Whole Blood Samples.

Skin reaction	No.	Avg 20 min. increase (μl O_2)	S.E.	Range
+	11	4.9	1.08	.8–14.4
—	8	1.5	.77	—1.8– 5.9

$S.E._{Diff.} = 1.33$ $p = .0106$

Fig. 2 represents the average response of duplicate determinations from 11 positive and 8 negative skin reactors.

The transitory effect is in agreement with the findings of Strauss and Stetson(4). Table I indicates the results of statistical analysis. The small and transient increase noted in the positives as compared to the negatives has a 1% level of significance. Although in skin reactions of the delayed hypersensitivity type, immunologically competent cells of the lymphocytic series have been the primary target of attention, they are not phagocytic, at least not in the usual sense. They may "imbibe" protein macromolecules and perhaps soluble antigen-antibody complexes by pinocytosis however. The dramatic increase in oxygen uptake due to phagocytosis (up to 46.6 μl/2 ml blood in 30 minutes) reported by Krenis and Strauss(3) would indicate that the increase noted in the positive skin reactors could be explained on the basis of increased phagocytic activity of the polymorphonuclear leucocytes in the peripheral blood. On the other hand, it is entirely possible the observed effect demands participation of both lymphocytes and polymorphonuclear leucocytes since both are plentiful in peripheral blood. To assess roughly the degeneration of the leucocytes during the 3-4 hour experimental period, a polymorphonuclear, lymphocyte ratio was obtained as follows. Wright stained blood smears made before and from the Warburg flasks upon completion of the manometric measurements were examined. Only neutrophils and lymphocytes were tabulated until 100 cells had been counted. The average ratios so obtained are indicated in Table II.

Since degranulation of polymorphonuclear

TABLE II. Average Leucocyte Ratios.

Skin test	No.	Wright's PMN/lymph before	after	PMN % degran
+	(10)	1.56	1.39	33
		Difference –.17		
—	(8)	1.52	.98	38
		Difference –.54		

leucocytes accompanying phagocytosis has been described(7), the per cent degranulation was estimated from counts of Wright's stained blood smears made upon completion of the Warburg run. There is very little difference between positive skin reactors and negative skin reactors in this respect (Table II). Under the conditions described, no evidence of increased phagocytosis in positive skin reactors was obtained.

Comparable observations on a duplicate set of blood smears using the fluorochrome Acridine Orange according to the method of Bertalanffy(6), corroborated the Wright's stain observations with respect to leucocyte ratios. Degranulation of polymorphonuclear leucocytes could not be estimated because the granules do not take up the fluorochrome. No attachment of coccidioidin to either polymorphonuclear leucocytes or mononuclear cells was evident.

Summary. Results of Warburg studies on 22 whole blood samples from positive skin reactors and 16 whole blood samples from negative skin reactors indicate a transient small but statistically significant increase in oxygen consumption upon *in vitro* addition of Smith's Lot 64D4 coccidioidin.

Studies of blood leucocytes before and after addition of coccidioidin using both the fluorochrome Acridine Orange and Wright's staining technic show no differences attributable to the coccidioidin addition or negative or positive skin reaction.

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Action of Pentolinium on Cardiac Output and Other Hemodynamic Parameters in the Rat.* (28454)

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There have been various suggestions as to the mechanism by which ganglionic blocking agents lower blood pressure. Some investigators have found that cardiac output remains unchanged after ganglionic blockade and, therefore, attribute blood pressure reduction to reduced total peripheral resistance. Others have shown that cardiac output decreases markedly, with total peripheral resistance remaining unchanged or being increased(1). Of those who find a reduced cardiac output, some consider this to be a result of reduced venous return(2-5), others believe

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