tions produced higher antitoxin titers than any other combination tested. There appears to be an inverse correlation between the circulating antitoxin at the time of challenge and the subsequent eosinophil responses. Animals with high antitoxin titers have fewer eosinophils in the inflammatory exudate. These results do not support the concept that the local eosinophilia is due to antigen–antibody reactions per se, but suggest instead that the local eosinophilia may be inhibited by the presence of antibody at the time of challenge.

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The Effects of Thiocyanate on Mitochondrial Respiration* (33414)

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The thiocyanate ion, SCN^-, has been extensively employed as a reversible inhibitor of gastric acid secretion. We have reported cytochrome redox changes associated with SCN inhibition in bullfrog gastric mucosa (1) and, since we found no SCN^- inhibition of oxygen consumption in tissue mince, concluded that the primary action of SCN^- was on the acid secretory mechanism. However, since Forte and Davies (2) have reported SCN^- inhibition of oxygen consumption in intact mucosal sheets it seems desirable to determine whether SCN^- can inhibit respiration in isolated, functionally intact mitochondria.

Materials and Methods. Attempts to prepare mitochondria from bullfrog gastric mucosae and bullfrog liver were unsatisfactory, as judged by the P/O ratio and respiratory control ratio of the particles obtained. Therefore, a standard rat liver mitochondria preparation (3) was employed, in which the mitochondria were reasonably intact by these criteria.

The final mitochondrial pellet was suspended in 0.25 *M* sucrose–0.01 *M* phosphate

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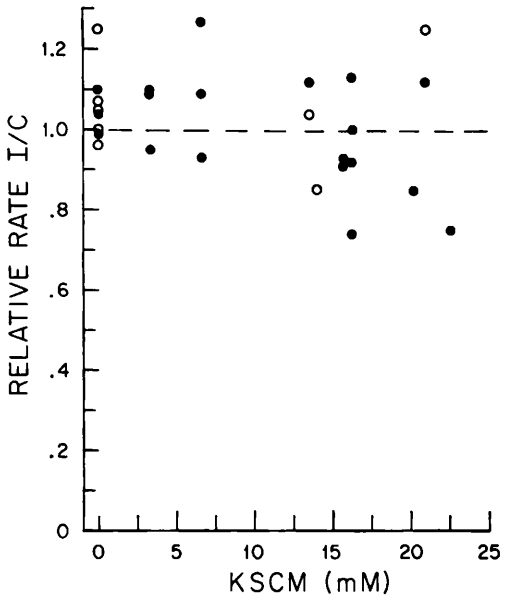

FIG. 1. Lack of inhibition of oxygen consumption by SCN (in range of 1–22 mM) in rat liver mitochondria. The control rate (C) was determined in the presence of 3.33 mM substrate and an initial concentration of 0.33 μM ADP (1 μM addition). The inhibited rate (I) was determined shortly after simultaneous addition of SCN and another 1 μM of ADP. (○), mitochondria using succinate as respiratory substrate; (●), α-ketoglutarate substrate.

buffer, pH 7.1, and all determinations were made in this medium. Oxygen consumption was measured at room temperature with a membrane-covered oxygen electrode fitted to a closed Lucite chamber. The maximum ADP-stimulated respiratory rate (C) was compared to the rate under the same conditions with added SCN^- (I) and the result expressed as a ratio (I/C).

Results. As an inhibitor of acid secretion in frog gastric mucosa, 2 mM SCN causes an inhibition of 50%, and inhibition is essentially complete at 10 mM (4). This is in marked contrast to the effect of SCN^- on mitochondrial respiration, as shown in Fig. 1. There was no significant respiratory inhibition at concentrations up to 22 mM, which makes it very unlikely that the effect of SCN^- on intact mucosal preparations is produced by an inhibition of mitochondrial respiration.

It is possible that the cells can concentrate SCN^-, and the concentration around the mitochondria in the tissue-sheet preparations might be higher than that in the bathing fluid. However it should be pointed out that SCN^- rapidly inhibits H^+ secretion (latent period is about 1 min) and that removal of SCN^- results in a rapid restoration of H^+ secretion. It is therefore difficult to believe that the intracellular SCN^- concentration could reach levels much above the ambient concentration in such a short time. We nevertheless explored the effect of high SCN^- concentrations in the mitochondrial experiments, realizing that at high SCN^- concentrations, the interpretation of any effects observed might be complicated by the osmolarity and ionic strength changes accompanying such large SCN^- additions.

Higher concentrations did inhibit oxygen consumption but the interpretation is indeed far from simple, as can be shown by examination of a typical oxygen polarographic trace of a SCN^- inhibition experiment at high concentrations (Fig. 2). After establishing the control rate, the addition of SCN^- to a final concentration of 46.5 mM inhibited the following ADP-stimulated rate by about 50%. Note that upon subsequent addition of ADP there is less inhibition, and that this is continued for still another addition. There is no significant change in the ratio of P_i este-

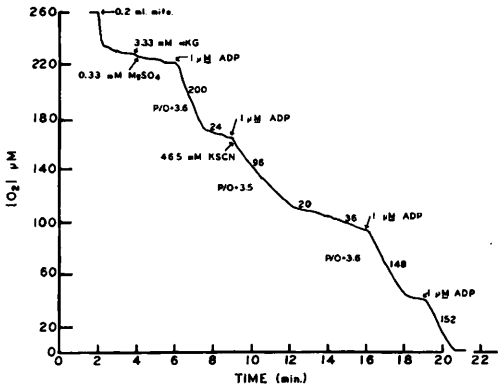

FIG. 2. Polarographic record of the inhibition of oxygen consumption in rat liver mitochondria by high SCN^- concentrations. Relative respiratory rates are indicated by the values near the trace. The addition of $MgSO_4$ had little if any effect, and was discontinued in later experiments. Note the increased rate following the third ADP addition.

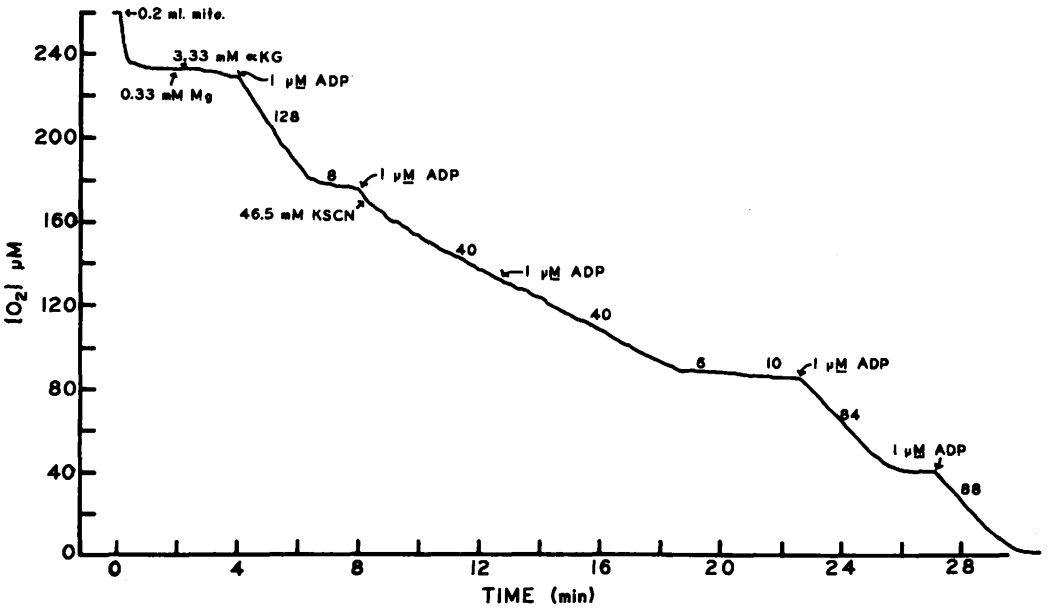

FIG. 3. Same as Fig. 2, but mitochondrial suspension diluted to enable longer times to be used. Note the lack of change of rate in the 10 min following the addition of KSCN (min 8–18) but the pronounced recovery after 4 min (18–22) in State 4.

rified to oxygen consumed (P/O ratio), as calculated from these data (5).

Further investigation showed that this recovery from inhibition occurred only under State 4 conditions (6), and was not a function of time alone. Figure 3 shows an example of an experiment in which the State 3 condition was prolonged by successive additions of ADP. There was no rate increase in the 10-min duration of the State 3 condition. However, 4 min in State 4 following this is sufficient to cause significant recovery. These results indicate that the recovery process requires the State 4 condition, that is, substrate, oxygen, and ATP. In Figs. 2 and 3, the ATP is generated by the mitochondria from ADP; added ATP is equally effective.

The mechanism of this release from inhibition is not clear. The analysis of the suspending medium at various times during the run shows no change in SCN^- concentration during the recovery period. The recovery process, therefore, involves a change in the mitochondrial reaction to constant SCN^- in the bathing fluid.

Determination of an inhibition curve for high SCN^- concentrations thus requires standardization of the procedure. We have elected to compare rate *I* under State 3 conditions just after SCN^- addition (but before recovery) with the rate *C* on the previous ADP addition in the absence of SCN. When this is done, a graph of inhibition (*I/C*) vs SCN^- concentration can be constructed, as shown in Fig. 4. It should be clear that the concentrations required for 50% inhibition of mitochondrial respiration are greatly in excess of the 2 m*M* required for inhibition in the *in vitro* mucosa.

Discussion. Forte *et al.* (7) have recently reported similar experiments with mitochondria from rabbit gastric mucosa. With their techniques, measuring the esterification of $^{32}P_i$, they report 40% inhibition of $^{32}P_i$ incorporation at 83 m*M* SCN^-, and 50% inhibition of the oxygen consumption of ADP-stimulated mitochondria by 40–50 m*M* SCN^-. One would calculate from these data that the P/O ratio must increase in this range of SCN^- concentration, which is not in agreement with our findings. Using the polarographic determination of P/O ratio, we do not find any significant P/O change even with high SCN^- concentrations.

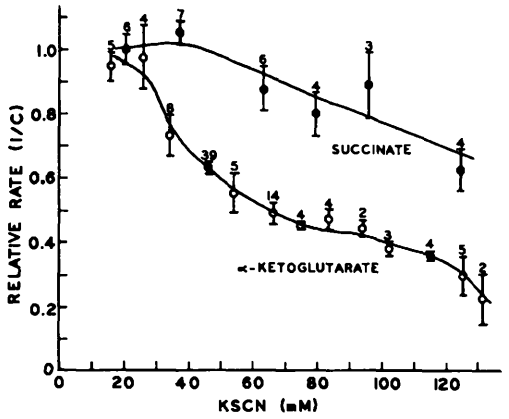

FIG. 4. Inhibition of oxygen consumption by high concentrations of SCN. Conditions are as given for Fig. 1. To enable averaging of data, the results for each 10 m*M* change in KSCN concentration have been grouped, and the average value of *I/C* plotted against the average KSCN concentration for that group. Bars represent ± one SE; the numbers are the number of determinations. Lines are drawn by eye.

If the inhibition of acid secretion in gastric mucosae is to be attributed to a direct effect of SCN^- on mitochondrial respiration, these experiments require either: (a) that the mitochondria in the gastric mucosa are more than 10 times as sensitive to SCN^- than are rat liver mitochondria, or (b) that a rapid and efficient mechanism exists for concentration SCN^- in the cytoplasm of the tubular cells. In addition, the effects of SCN^- are continued in the stomach, and show no recovery with time as is seen in isolated mitochondria. The concentration of SCN required to inhibit respiration of isolated mitochondria is more than 10-fold greater than that required to inhibit gastric acid secretion.

It therefore seems unlikely that the effects of SCN^- on the gastric acid secretion are explained by inhibition at the mitochondrial level as has been suggested. It appears that effects should be sought at the level of the acid secretory mechanism, as originally postulated.

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Effects of Inanition on a Nutritional Index and Cold Tolerance in Cats* (33415)

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Alterations in the ability to regulate body temperature have been shown to result from changes in the nutritional state (1–4). Thus, some measure of fatness or thinness is needed to insure that the changes found during cold exposure can be attributed solely to the environment. We have attempted to determine if the approach used by Von Pirquet (5) in the human or its adaptation to the dog by Cowgill and Drabkin (6) would be useful in the cat.

Methods. Changes in response to cold exposure were followed in six cats (two males

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