large amounts, 5 classes of proteins having a sedimentation constant above 7S.

Summary. When a rabbit antiserum to human macroglobulin was reacted with human sera by immunoelectrophoresis, several distinct macroglobulin precipitin lines were revealed. Internal to the major beta-2M line all sera showed a second, and some a third, line. A fourth macroglobulin line appeared opposite the serum source in most sera examined. The major macroglobulin line was forked at its anodal end in some sera. The major macroglobulin line had a single curvature when produced by the rabbit antiserum used in most of these experiments, but had a double curvature when produced by a horse antiserum. These findings could be due to multiple antigenic sites on macroglobulins of a single molecular species, or to antigenically distinct macroglobulin molecules.

1. Kunkel, H. G., Fudenberg, H. H., Ovary, Z., *Ann. N. Y. Acad. Sci.,* 1960, v86, 966.

2. Burtin, P., Hartmann, L., Heremans, J., Scheidegger, J. J., Westendorp-Boerma, F., Wieme, R., Wunderly, Ch., Fauvert, R., Grabar, P., *Rev. Franc. Etudes Clin. et Biol.,* 1957, v2, 161.

3. Habich, H., Hässig, A., *Vox Sanguinis,* 1953, v3, 99.

4. Westendorp-Boerma, F., Mandema, E., *J. Lab. and Clin. Med.,* 1957, v49, 358.

5. Korngold, L., vanLeeuwen, G., *J. Exp. Med.,* 1957, v106, 467, 477.

6. Grunbaum, B., Dong, L., *Nature,* 1962, v194, 185.

7. Fessel, W. J., *Acta Med. Scand.,* 1962, v173, suppl. 391, 1.

8. Scheidegger, J. J., Buzzi, C., *Rev. Franc. Etudes Clin. et Biol.,* 1957, v2, 895.

9. Wallenius, G., Trautman, R., Kunkel, H. G., Franklin, E. C., *J. Biol. Chem.,* 1957, v225, 253.

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Oxidative Responses *in vivo* of 2,4-Dinitrophenol and Carbonyl Cyanide-p-Trifluoromethoxyphenylhydrazone. (28393)

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A recent report has indicated that ring-substituted derivatives of carbonyl cyanide phenylhydrazone are potent uncouplers of oxidative phosphorylation in both animal and plant mitochondrial systems(1). The most effective of the compounds reported was carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (p-CF_3O-CCP), reported to be about 1000 times as active as 2,4-dinitrophenol (DNP) *in vitro*. It was, therefore, of interest to measure the general oxidative response of intact laboratory animals to p-CF_3O-CCP.

The present report contains results obtained from mice and rats in a comparative study of metabolic effects of DNP and p-CF_3O-CCP. Measurement of both $C^{14}O_2$ resulting from oxidation of C^{14}-2-acetate and total expired CO_2 was used to determine general oxidative responses to uncoupling agents and as an indication of metabolic rate (MR) respectively. Stimulatory effects of DNP have been observed using similar methods(2) and oxidative uncoupling effects of DNP are well established(3).

Materials and methods. Ad libitum fed male albino mice and rats were used. Mice weighed 27-32 g (Rockland Farm Swiss mouse ancestry) and rats weighed 165-175 g (Charles River). Fresh solutions of uncoupling agents were made daily and injected intraperitoneally (IP) into mice or rats in 0.2 ml volume; control animals received the appropriate vehicle alone. Solutions of DNP in 0.1 N $NaHCO_3$ were given at a dose of 15 mg/kg while p-CF_3O-CCP* (11 mg/kg) was administered in methyl cellulose solution (0.25% aqueous vehicle). These doses represented approximately 20% of the acute

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TABLE I. Effects of DNP and p-CF_3O-CCP on Exhaled CO_2 and Oxidation of C^{14}-2-Acetate by Mice and Rats.

Group	C^{14} dose oxidized (%)	40 min. exhaled CO_2 mM CO_2*	Specific activity (cpm/mM)†
Nonfasted mice			
1. Controls (5)‡	13.5 ± 1.2‖	9.1 ± .2	978 ± 78
2. DNP	18.5 ± 1.0	11.5 ± .3	1072 ± 78
3. Controls	14.4 ± .3	7.2 ± .2	1324 ± 28
4. p-CF_3O-CCP	15.5 ± .3	7.3 ± .5	1420 ± 74
Nonfasted rats			
5. Controls (5)§	10.8 ± .8	8.9 ± .5	674 ± 52
6. DNP	14.5 ± 1.2	15.3 ± .2	522 ± 46
7. Controls	10.7 ± 1.0	8.4 ± .6	734 ± 106
8. p-CF_3O-CCP	16.1 ± 2.0	25.6 ± 2.0	352 ± 64

* Millimoles of CO_2 at STP.
† C^{14} counts/minute/millimole CO_2 exhaled.
‡ Number of runs (3 mice/run) for groups 1-4.
§ Number of runs (1 rat/run) for groups 5-8.
‖ Standard error of mean.

LD_{50} of compounds given IP to other nonfasted mice. C^{14}-2-acetate, sodium was dissolved in 0.9% saline and injected IP 40 minutes after the test compounds or vehicle were given. Each mouse received 0.05 μc C^{14}/0.2 ml and each rat received 0.125 μc C^{14}/0.5 ml.

A group of 3 mice or a single rat (control and treated of equal weights alternating throughout the day) was placed in a glass metabolic chamber for 40 minutes and total exhaled $C^{14}O_2$ and CO_2 measured by a continuous gas analyzer† utilizing an ion chamber electrometer and an infrared analyzer to monitor the respective gases. Values for expired $C^{14}O_2$ were calculated as counts per minute (cpm) based on results from a gas flow counter used for standardization where 1 μc = 4.4×10^5 cpm. Values for expired CO_2 were calculated in millimoles (mM) on the basis of standard conditions of temperature and pressure (STP) compared to known CO_2.

Results and discussion. Both uncoupling agents increased respiratory CO_2 output of rats whereas only DNP produced an increase in mice (Fig. 1). From calculations based on areas under curves, p-CF_3O-CCP produced an effect in rats approximately 3 times that of DNP.

p-CF_3O-CCP, at a dose of 11 mg/kg which was barely sublethal for mice, did not appear to increase oxidation of C^{14}-acetate or total exhaled CO_2 by mice (Table I). In rats, p-CF_3O-CCP increased C^{14}-acetate oxidation (50%) from 10.7 to 16.1% of dose injected. However, exhaled CO_2 was increased to a greater extent, (204%) from 8.4 to 25.6 mM, thereby lowering specific activity (52%) from 734 to 352 cpm/mM for rats. DNP, at a dose of 15 mg/kg, increased oxidation of C^{14}-acetate about the same amount in rats and mice, 34 and 37% respectively. Since DNP increased total exhaled CO_2 of rats (72%) from 8.9 to 15.3 mM and of mice (only 26%) from 9.1 to 11.5 mM, DNP lowered specific activity for rats about 22% but did not significantly affect specific activity for mice. Hence, DNP appeared to be more active in rats than mice and differed from p-CF_3O-CCP in that it was effective in both species.

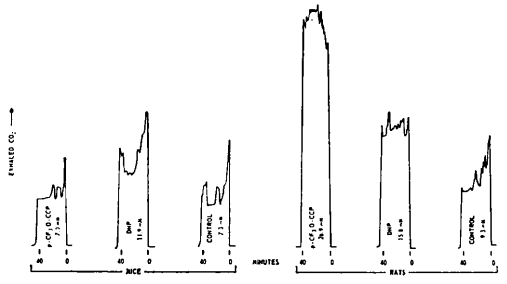

FIG. 1. Typical effects of DNP and p-CF_3O-CCP on millimoles of expired CO_2 from nonfasted rats and mice.

† Model 2000, General Measurements, Inc., Garnerville, N. Y.

Since p-CF_3O-CCP was reported to be highly active as an uncoupling agent in mouse liver mitochondrial preparations(1), a further attempt to observe stimulatory effects of p-CF_3O-CCP in intact mice was made. The dose was increased from 11 mg/kg to 15 mg/kg but this slightly higher dose of p-CF_3O-CCP killed enough mice so as to prevent measurements of MR. Further experimentation is necessary in order to determine the reason for lack of demonstrating increased MR with p-CF_3O-CCP in the intact mouse. Furthermore, since higher doses of p-CF_3O-CCP killed mice without gross indications of increased MR, the primary toxic effect responsible for death of mice may not be associated with uncoupling of phosphorylation *per se*.

Summary. In rats, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (p-CF_3O-CCP) was more potent than 2,4-dinitrophenol (DNP) in causing marked oxidative and expiratory effects but was without apparent effect in mice at an equivalent dose. Toxicity of p-CF_3O-CCP in mice prevented further observations since increasing the dose proved lethal. DNP was active in both mice and rats.

1. Heytler, P. G., Prichard, W. W., *Biochem. Biophys. Res. Comm.*, 1962, v7, 272.

2. Miller, W. L., Krake, J. J., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 585.

3. Brody, T. M., *Pharmacol. Rev.*, 1955, v7, 335.

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Studies on Soluble Antigenic Substances Isolated from Human Liver. (28394)

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Tissue homogenates, as well as specific cellular components from a variety of mammalian organs, are reactive in complement fixation (CF) tests with serum substances from patients with systemic lupus erythematosus (S.L.E.) and other diseases(2-10). These reactive tissue components include cellular nuclei, mitochondria, microsomes, and soluble cytoplasmic factors isolated from human, rat, rabbit, and calf liver(2,6-8), as well as fractions from calf thymus(5-7), human kidney(7), and thyroid(9) and human and rabbit pancreas(10). The reactivity of cellular nuclei and deoxyribonucleic acid-protein complexes in CF tests has been well documented with sera from persons with S.L.E.(5-7,11). In addition, substances in the cytoplasm of cells have also been shown to react with sera from patients with S.L.E. (2,6-8). These cytoplasmic substances, however, have not been adequately characterized. This report concerns their isolation, fractionation, and chemical characterization.

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Materials and methods. Human liver obtained at autopsy from young accident victims 6-12 hours post mortem was frozen and stored at –60°C. For antigenic isolation, each gram of liver was extracted for one minute in a Virtis homogenizer with either 9 ml of 0.15 M sodium chloride or 0.44 M sucrose. All processes were conducted at 1° to 3°C unless otherwise indicated. The cell nuclei and tissue debris were removed by immediate centrifugation (10 min. at 700 × *g*) of the homogenate. The sediment was washed once with the extractant and the wash combined with the supernatant solution. Sucrose was the extractant of choice when cellular constituents were desired, and these were separated by differential centrifugation(7). For isolation of the soluble fraction, the clear supernatant solution remaining after centrifugation for 1 hr at 105,000 × *g*, 0.15 M sodium chloride was used.

The soluble fraction was further fraction-