Table 1. Effect of vasectomy on hepatic drug metabolism in rats*

Drug substrate	Control (nmoles/min g liver)	Vasectomized (nmoles/min g liver)	Change (%)
Aniline	7.52± 0.55 (5)	10.36±0.57 (5)**	+ 38
Aminopyrine	104.9 ±12.83 (5)	131.16±4.51 (5)***	+ 25

*The results are expressed in terms of the metabolic product formed (see Materials and methods) and the numbers given are the mean ± SEM (number of animals). ** $p < 0.01$ versus control. *** $p < 0.07$ versus control.

Table 2. Effect of vasectomy on hepatic drug metabolism in guinea-pigs*

Drug substrate	Control (nmoles/min g liver)	Vasectomized (nmoles/min g liver)	Change (%)
Aniline	6.55±0.67 (6)	6.85±0.34 (7)	+ 5
Aminopyrine	43.05±2.25 (6)	47.07±1.75 (7)	+ 9

*The results are expressed the same as in table 1.

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strates for drug metabolism reactions in 10,000 × g – 10 min liver supernatant preparations as described by Mazel[3]. Metabolism of aminopyrine and aniline were determined by assaying the formation of formaldehyde and p-aminophenol, respectively, as previously described[3] except that 20% trichloroacetic acid was used to stop the reactions. The significance of difference between means was established by the Student's t-test.

Results and discussion. Vasectomy significantly increased the in vitro metabolism of aniline by rat liver homogenates (table 1). This increase is similar to and confirms the observation of Esterday et al.[2] who found that vasectomy of rats increased the in vitro metabolism of p-chloro-N-methylaniline. Both aniline and p-chloro-N-methylaniline exhibit type II binding spectra with hepatic cytochrome[4] even though they undergo different types of metabolism (aromatic hydroxylation and N-demethylation, respectively). By contrast, the metabolism (N-demethylation) of aminopyrine (a type I compound)[5], although appearing to be increased, was not significantly affected by vasectomy (table 1). These results suggest that vasectomy may affect drug metabolism through a mechanism that alters specific substrate-cytochrome interactions.

The results in table 2 show that vasectomy of the guinea-pig had no effect on the hepatic metabolism of either aniline or aminopyrine. These findings suggest that the effects of vasectomy on hepatic drug metabolism are species specific and raise the question of which laboratory species is an appropriate model for indicating possible metabolic consequences of vasectomy in man. The results of the present study emphasize the need for more extensive studies on the relationship between vasectomy and hepatic drug metabolism in both animal model systems and man.

Radioimmunoassay of polyacrylamide[1]

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Summary. Antiserum to polyacrylamide has been produced in rabbits. It can be used in a sensitive radioimmunoassay for quantitation of polyacrylamide at very low concentrations. This novel approach to the analysis of a synthetic, biologically inactive, hydrocarbon polymer has potentially widespread implications, for example, in the downstream measurement of flocculating agents used in water purification treatments.

Radioimmunoassay (RIA) was developed for detection of biochemicals at very low concentrations in biological fluids[2]. Peptide, protein and steroid hormones, digitalis alkaloids, morphine, LSD and other drugs have been measured by RIA. This report illustrates the adaptability of the technic to a different class of substances. We have developed an RIA for polyacrylamide, a nonbiological, nonnaturally occurring compound, one use of which is as a water purifying agent (Dowfloc™) on the basis of its flocculant properties. There is a need to determine the downstream concentration of polyacrylamide in the range of parts per million to parts per billion. Conventional analytic methods for polymers in aqueous solution are not sufficiently sensitive or selective to meet this requirement. Polyacrylamide resin 164 (Dow Chemical Company) of mol. wt of approximately 200,000, with 3.4% of the amide groups hydrolyzed to carboxylic acid, was exhaustively dialyzed to remove low molecular weight materials (PAA). Polyacrylamide-bovine serum albumin conjugate (PAA-BSA conjugate) was prepared by reacting the carboxylic acid groups on the polymer with the amine groups on the BSA (Miles Laboratories) via the carbodiimide method[3], as follows: Approximately equimolar amounts (2.0 g PAA, 0.7 g BSA) were dissolved in 50 ml of water and the pH adjusted to 4.7 with HCl. Approximately 150 mg of 1-ethyl-3-dimethylaminopropyl carbodiimide (Aldrich Chemical Co.) was added, and the reaction mixture was stirred for 48 h, then exhaustively dialyzed against deionized water and lyophilized. Approximately 2 g of the reaction product was recovered. Samples taken from the reaction mixture at 0, 4, 8, 24 and 48 h were analyzed via aqueous gel permeation chromatography on Sephadex G-150, eluting with 0.2 N NaCl and detecting with a Waters R-400 refractometer. Both the PAA and the PAA-BSA complex were completely excluded from the gel and eluted at V_o; however, BSA was resolved and eluted at $V_o + 0.4\ V_i$. The formation of the product was inferred from the disappearance of the

BSA starting material (table 1). The data indicate a product of approximately 70% (w/w) PAA-BSA. Polyacrylamide-^{14}C, 3.7% hydrolyzed, was polymerized from ^{14}C-labelled acrylamide (New England Nuclear) by heating a 10% solution at 70 °C under N_2, diluting to 0.5%, adjusting pH to 11.2 with 2 N NaOH and heating at 80 °C for 1 h under N_2. The product was cooled, neutralized and dried. Specific activity: 345 μCi/g.

Equal volumes of Freund's adjuvant and a 1.0% solution of either PAA or PAA-BSA conjugate in saline were mixed to form an emulsion. Albino New Zealand rabbits were immunized by injection of 2 ml of emulsion in multiple s.c. sites (10 mg PAA or PAA-BSA conjugate per total injection) every 2 weeks for 3 months, and approximately every month for a total of 8 months. In the first 3 injections Freund's complete adjuvant was employed, all subsequent booster injections were in Freund's incomplete adjuvant. Test bleedings were obtained from the ear vein at intervals during the schedule. The potency of the antiserum was checked by incubating ^{14}C-PAA with either the antiserum (as for zero standard in the radioimmunoassay protocol, table 2) from the immunized rabbit or the control rabbit serum (obtained before the immunization schedule).

Table 1. Relative concentration of BSA in the PAA-BSA reaction mixture

Time (h)	BSA* (%)
0	100
4	60
8	40
24	30
48	30

*Normalized to 100% at t = 0.

Table 2. Radioimmunoassay protocol (additions are in μl)

	Each working standard	^{14}C-PAA	Diluted antiserum (normal serum)	PBS-NRS	Goat anti-rabbit
Nonspecific	–	100	(100)	800	100
Zero standard	–	100	100	800	100
Other standards	100	100	100	700	100

Table 3. Ability of antisera to bind labeled polyacrylamide. Percent of labeled polyacrylamide bound specifically by antisera

Rabbit	Dilution	Weeks 6	14	16	19	23	26	29	30	32	35	38
255	1:10	47	47	66	83	90	90	70	70	45	48	29
	1:100	4	12	18	16	19	21	10	19	7	8	4
256	1:10	70	37	50	74	69	69	42	54	32	32	12
	1:100	13	9	13	11	9	11	3	8	4	2	1
257	1:10	10	41	55	63	47	53	61	35	16	35	11
	1:100	–	9	10	6	5	4	14	2	1	4	1
258	1:10	13	24	34	–	70	77	82	63	35	40	21
	1:100	0	5	7	–	12	16	17	13	6	5	3
259	1:10	63	44	died								
	1:100	12	12									

The radioimmunoassay was performed as follows: polyacrylamide was dissolved in phosphate buffered saline, pH 7.6, then a series of standards ranging from 1 to 100 μg/ml (i.e., 1–100 ppm) was made by serial dilution. Each standard (100 μl) was incubated with 100 μl (1 μg) of ^{14}C-PAA and 100 μl of an appropriate working dilution of antiserum in a total volume of 1.0 ml phosphate buffered saline containing 1% normal rabbit serum (PBS-NRS) for 72 h at 4 °C. Goat anti-rabbit serum (100 μl) was added to precipitate the antigen-antibody complex during 18–24 h incubation at 4 °C. After centrifugation, the precipitates were redissolved in 0.5 ml of 0.1 N NaOH, transferred to counting vials, and 10 ml scintillation fluid (Clinical Assays, Inc.) was added. After equilibration in the cold and dark, samples were counted for 20 min in a liquid scintillation spectrometer set for ^{14}C. Appropriate background (NaOH and scintillation fluid) and total count (label, NaOH and scintillation fluid) controls were set up at the same time.

A positive antibody response was obtained from all rabbits immunized with PAA-BSA conjugate. The percent of counts added to the system that were bound by antiserum (minus the percent bound nonspecifically by the control serum) is shown in table 3 at 2 levels of antiserum dilution. Antiserum activity was evident at 6 weeks, rising to a maximum at about 6 months. From the 2 rabbits with highest titer at 6 months, antiserum at dilutions of about 1:100 (final dilution in total incubation volume 1:1000) gave initial binding in the radioimmunoassay of 17 and 21% (corrected for nonspecific binding) of added counts. Another 5 rabbits, injected with polyacrylamide not conjugated to bovine serum albumin, did not show any measurable antiserum activity after 3 months of injections. Using the highest titer antiserum, a balanced radioimmunoassay system was set up. A typical standard curve is shown in the figure. The initial 23% of antibody-bound labeled polyacrylamide is progressively inhibited by increasing amounts of unlabeled PAA over the range of 1–100 μg/ml. This indicates that 1–100 ppm water soluble polymer in effluent streams could be directly measured in 100 μl of sample.

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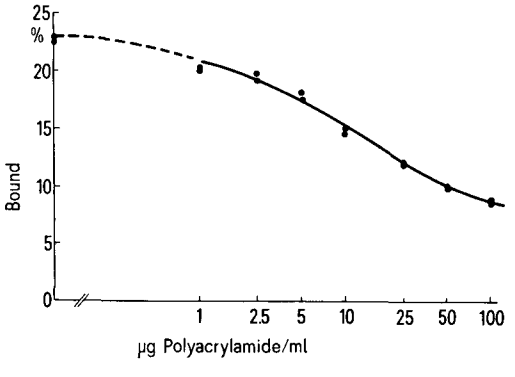

Standard curve for measurement of polyacrylamide. Working dilution of antiserum was 1:50 (final titer 1:500); antiserum obtained from rabbit 255 after $5^1/_2$ months into the immunization schedule.

Polyacrylamide is not antigenic in itself, but when conjugated to bovine serum albumin, antibody capable of specifically binding radioactively labeled PAA is elicitable. Its affinity appears to be quite small; however, material of higher specific activity would increase the sensitivity, which in turn would increase the titer of the antibody proportionately. A final dilution of 1:5000 or more would be entirely practical for the routine analysis of polyacrylamide in effluent waters. The results indicate that RIA may prove useful in analysis of other water soluble synthetic polymers that enter the environment, such as electroconductive resins or polyethyleneimine, and could be extended to analysis of samples of downstream effluents.

Proteolysis in dystrophic hamster diaphragm and abdominal muscle

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Summary. Proteolysis, as measured by tyrosine release, was estimated in abdominal and diaphragm muscle of hamsters. There did not appear to be a difference between dystrophic and control hamsters.

Wasting of muscle is one of the characteristics of muscular dystrophies. In spite of research interest in these diseases for several years, the etiologies are still unknown. Early reports by Weinstock, Epstein and Milhorat[2], and others led to the suggestion that abnormal protein breakdown might be an important factor in the progress of some of the muscular dystrophies. Thus the objective of this study was to determine whether increases in proteolysis could be detected prior to detection of other changes described in the literature. In this communication we report the degradation of protein in muscle of young hamsters.

Table 1. Tyrosine release by abdominal and diaphragm muscles of dystrophic hamster

μg Tyrosine release/mg noncollagen protein in 2 h				
Age	Diaphragm		Abdominal	
(days)	Control	Dystrophic	Control	Dystrophic
3	0.77 ± 0.24 (6)	0.84 ± 0.38 (10)	0.88 ± 0.17 (6)	0.98 ± 0.20 (10)
7	1.04 ± 0.46 (6)	0.62 ± 0.25 (6)	0.89 ± 0.09 (6)	0.89 ± 0.15 (6)
11	0.76 ± 0.14 (6)	0.46 ± 0.32 (6)	0.89 ± 0.14 (6)	0.71 ± 0.27 (6)
15	0.51 ± 0.13 (6)	0.63 ± 0.12 (6)	0.53 ± 0.02 (6)	0.72 ± 0.20 (6)

Incubations were conducted in Krebs-Ringer bicarbonate solution which contained 0.5 mM cycloheximide. Values given are means ± SD; numbers in brackets indicate the number of animals.

Table 2. Tyrosine release by dystrophic hamster muscle in the absence of cycloheximide

μg Tyosine release/mg noncollagen protein in 2 h				
Age	Diaphragm		Abdominal	
(days)	Control	Dystrophic	Control	Dystrophic
3	1.36 ± 0.83 (6)	0.74 ± 0.36 (10)	0.84 ± 0.54 (6)	0.97 ± 0.75 (10)
7	0.58 ± 0.29 (7)	0.38 ± 0.17 (7)	0.44 ± 0.09 (6)	0.51 ± 0.10 (5)
11	0.39 ± 0.09 (7)	0.59 ± 0.43 (6)	0.40 ± 0.12 (7)	0.48 ± 0.33 (6)
15	0.43 ± 0.03 (5)	0.85 ± 0.58 (8)	0.45 ± 0.02 (5)	0.91 ± 0.76 (8)
20–40	0.43 ± 0.14 (7)	0.60 ± 0.17 (6)	0.56 ± 0.25 (8)	0.62 ± 0.18 (7)

Conditions were the same as for table 1 except that cycloheximide was excluded.

Materials and methods. The hamsters used for these experiments were UM-X7.1 and the controls were Syrian Golden Hamsters; both sexes were used. Because of the young age of the animals required, they were all bred and raised in the animal quarters of Queen's University. Hamsters were sacrificed by decapitation and their diaphragms and abdominal muscles were removed as quickly as possible. The rate of tyrosine release from the muscles was then estimated by the method of Fulks, Li and Goldberg[3] to provide an index of proteolysis. Briefly the tissues were preincubated (37 °C) in 3 ml of Krebs-Ringer bicarbonate solution (KRB) for 30 min and then incubated in a further 3 ml of KRB for 2 h. After removing the tissues the tyrosine contents of the incubation media were determined by the method of Waalkes and Udenfriend[4]. This procedure involves reacting tyrosine with 2-nitrosonaphthol and measuring fluorescence intensity. Protein as noncollagen protein was measured by the method of Lilienthal et al.[5], using bovine serum albumin as the reference.

Results and discussion. Homburger et al.[6] have reported that the earliest detectable morphological lesion in dystrophic hamsters occurs at about 20 days of age. Since we wished to learn whether alterations in protein degradation contributed to such observations it was necessary to examine animals younger than 20 days. Table 1 shows the rates of tyrosine release from diaphragms of animals as young as 3 days. These were the youngest animals which we could handle because of their small size. At any of the ages studied there was no difference between the dystrophic and control hamsters. These data are consistent with those of Goldspink and Goldspink[7] at the youngest age they used (30 days).

In the present studies experiments were conducted in the presence of cycloheximide to inhibit re-incorporation of tyrosine and also in its absence. Several reports in the last 2 years have indicated that the action of cycloheximide may not be simple blockade of protein synthesis as had been formerly assumed. For example, Woodside[8] found

1 Supported by the Muscular Dystrophy Association of Canada.

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