Comment. Raised medium strength traps HeLa cells in metaphase while allowing a normal G_2 population to enter mitosis and retarding progression of S phase cells. This contrasts with HUGHES'[1] finding in chick cells where progression was immediately halted. At the levels of hypertonicity employed (about 227 m*M* for HeLa cells) there was no evidence of prophase 'induction' or nuclear chromatin condensation[3]. Cells progressed normally through this stage of mitosis in expected numbers.

It has been clearly demonstrated that hypertonically collected metaphases remain readily reversible for 3–4 h but degenerate quickly if held for longer periods of time. Hypertonic treatment induces an immediate metaphase arrest, but, isotonically collected anaphases and telophases in hypertonic medium (and hypertonically collected metaphases returned to isotonic medium) show unusual nuclear behaviour after completing normal cytokineses which involves the lobulation of the nucleus often into 2 roughly equal parts before further lobulation.

Synchronization of cells by mechanical collection in metaphase[8] is aided by the technique of hypertonic treatment and gives good yield of nearly pure metaphase cells. At present the cause of metaphase arrest by hypertonicity is unknown. There is no evidence of spindle abnormalities but chromosomes tend to be supercondensed and more sticky, probably preventing their anaphase separation.[10]

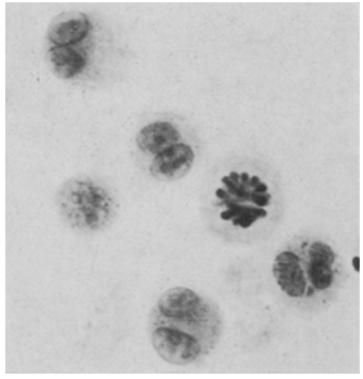

Fig. 6. Isotonically collected mitotic HeLa cells exposed to 227 m*M* hypertonic medium for 6 h. The metaphase shows heavy condensation of cromosomes into several groups. Cells which have reached interphase appear binucleate. Crystal violet. ×600.

Résumé. L'arrêt de la métaphase se produit dans les cellules de HeLa S-3 au contact d'un milieu de tonicité accrue (> 165 m*M*). L'accumulation optimale intervient à 277 m*M* lorsque du NaCl a été utilisé pour augmenter l'influence du milieu. Les effets de l'usage d'autres sels et d'autres types de cellules sont décrits. La dilution du milieu à 165 m*M* donne lieu à un arrêt synchrone du processus de la mitose dans les cellules et assure la conservation d'un bon synchronisme au cours de la division cellulaire suivante.

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10 April 1973.

[9] H. FAN and S. PENMAN, J. molec.Biol. *50*, 655 (1960).

[10] This work was supported by the Cancer Research Campaign and the Wellcome Foundation. Mrs. M. INGLIS and Miss W. ELMSLIE provided technical assistance.

A New Liver-Specific Autoantigen in the Rat: Delta Antigen

When inbred mice were immunized with allogeneic liver extracts, autoantibodies reacting with the liver-specific 'F antigen' were induced in certain strain combinations[1,2]. Since it seemed unlikely that the F antigen system reflected a situation unique to the mouse, we investigated the capacity of inbred rats to be similarly immunized.

Rats from inbred strains DA, Lewis and BN were used. Aqueous liver extracts from each strain were emulsified in complete Freund's adjuvant and injected every other week into syngeneic or allogeneic rats by the intraperitoneal route. Up to 5 injections were given. Serum samples were tested at intervals for the presence of precipitating antibodies by the Ouchterlony technique, liver extracts from each of the three strains serving as antigens.

Table I. Results of immunization of rats with rat liver extracts

Strain immunized	Stimulating liver extracts from DA	Lewis	BN
DA	− −	− −	− −
Lewis	**	− −	**
BN	**	+ +	− −

− −, No precipitating antibody detectable; **, weak precipitating alloantibody not reacting with Lewis extract (see text); + +, strong precipitating autoantibody reacting with all 3 rat liver extracts and defining the liver antigen Delta.

The results of these immunizations are shown in Table I. As in the mouse, even prolonged syngeneic stimulation (e.g., BN liver extracts injected into BN rats) never yielded precipitating antibodies. DA rats proved also refractory to allogeneic stimulation by either Lewis or BN extracts; this was reminiscent of the situation in BALB/c and DBA/2 mice which did not make precipitating antibodies upon stimulation with several mouse liver extracts[1]. Lewis rats, after immunization with either DA or BN extracts, did produce weak precipitating antibodies; these antibodies, however, were not autoantibodies, since they failed to precipitate syngeneic (Lewis) extracts. Rather, they appeared to recognize some alloantigen characteristic of strains DA and BN, and different from known rat allotypes. This system, for which no counterpart in the mouse is known, has not been studied in detail.

BN rats, upon stimulation with Lewis antigen, after 3 to 4 injections regularly produced strong precipitating antibody which reacted with all 3 liver extracts and had

[1] G. FRAVI and J. LINDENMANN, Nature *218*, 141 (1968).
[2] G. FRAVI, Path. Microbiol. *31*, 257 (1968).

Table II. Comparison of Delta and F antigens from rat liver

Property	Delta	F
Mol. weight (gel filtration)	around 58,000	around 65,000
pI (ion exchange chromatography)	lower than 7	around 7
Immuno-electrophoresis	mobility of α-globulin	mobility of β-globulin
Anion exchange chromatography	several molecular species	no detectable polymorphism
Temperature stability		
50 °C 10 min	inactivated[a]	little affected
−30 °C	decrease to $^1/_2$ of original activity within 15 h	unaffected by prolonged frozen storage
pH stability		
pH 5	inactivated	unaffected
pH 11	inactivated	little affected
Effect of enzymes		
DNAase	unaffected	unaffected
RNAase	unaffected	unaffected
α-Chymotrypsin	inactivated only when semipurified	crude extracts readily inactivated
Localization in liver cells	cytosol	cytosol
Distribution in various organs		
liver	large amount	large amount
kidney	small amount	small amount
other organs	not detectable	not detectable
Distribution of cross-reactive antigens in various species		
Mouse	yes	
Rat		yes
Cow	yes	yes
Man	yes	yes

[a] Inactivated = substance no longer precipitable by corresponding antisera in gel diffusion tests.

thus characteristics of a true autoantibody. This was further ascertained by showing that the serum from one animal precipitated an extract made from the liver of that same animal. The autoantigen involved will henceforth be called Delta. The exact immunological relationship between anti-Delta and the alloantibodies mentioned above (induced in Lewis rats by BN and DA extracts) has not yet been worked out.

Since the autoantibody-autoantigen system of rats was so strikingly similar to that found earlier in mice, it seemed important to compare the two antigens. Both were widely distributed in liver extracts of mammalian species, so that a meaningful comparison within the same species was possible. For instance, rat liver extracts, in addition to Delta, contained an antigen which strongly cross-reacted with F antigen from mouse liver[1], and mouse liver extracts, in addition to F, contained a Delta-like antigen. Immunologically, the 2 antigens appeared completely unrelated in all species tested (rat, mouse, cow and man), with undisturbed crossing of the precipitation lines in double diffusion studies. Nevertheless, physicochemical properties of the 2 antigens were grossly similar, as shown in Table II. (The data shown are similar to those of mouse liver F antigen[3,4].)

It therefore appears that autoantibodies to soluble liver antigens can be readily induced in 2 species, rat and mouse, provided allogeneic stimulation in the proper strain combination is arranged.

Zusammenfassung. Es werden einige Eigenschaften eines leberspezifischen Antigensystems der Ratte beschrieben. Es wird gezeigt, dass das entsprechende Antigen aus Lewis-Ratten in BN-Ratten einen universell kreuzreagierenden Autoantikörper induziert.

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[3] F. PESARO and H. KOBLET, Experientia 27, 235 (1971).
[4] F. PESARO and H. KOBLET, Experientia 27, 1106 (1971).

Murine Thyroiditis Induced by Neonatal Thymectomy

Since WITEBSKY and ROSE[1] reported an experimental induction of thyroiditis in rabbits by an immunizing procedure, allergic thyroiditis has been induced by the same treatment in various laboratory animals, including mice. With the establishment of the thymic role in cellular immunity, it has now been generally accepted that experimental allergic thyroiditis (EAT) is also dependent on the presence of the thymus, especially of thymus-derived lymphoid cells (T-cells). In chickens[2] and rats[3], neonatal thymectomy made the animals less responsive to the induction of thyroiditis.

On the other hand, spontaneous autoimmune thyroiditis (SAT) has been reported in dogs, chickens and rats. WICK et al.[4,5] and recently ROSE et al.[6], using the Obese