A comparison was made between the results obtained with the GBA method and those obtained with the ACT method. The DβH levels were considerably lower when assayed by the GBA method (Table). However, when the adrenal extract was diluted further the DβH levels as estimated by the GBA method remained constant, whereas when estimated by the ACT method the level declined. This result suggests that in concentrated extracts some unspecific bindings interfere with the DβH assay by the ACT method.

The low levels of DβH in the medulla oblongata and the virtually undetectable levels in the striatum as shown by the GBA method are consistent with the reported enzyme activity levels in these regions of the brain[5]. These results indicate that the GBA method is more specific than the ACT method.

One of the attractive features of the GBA assay is the relative ease of operation as compared to the ACT method. Once prepared, the glass-bound antibody can be stored and used for many assays, whereas the previously described method requires that each tube be coated with antibody separately. Another advantage is superior precision. Replicate assays by the GBA method yield mean values with less than 1% in the standard error, whereas the corresponding value for the ACT method is about 5%.

The simplicity of the GBA method would render it applicable for routine clinical assays of human serum DβH. Human DβH antibodies have been covalently bound to diazotized arylamine glass and studies on measurements of human serum DβH are in progress[6].

Résumé. Les auteurs décrivent une méthode radioimmunologique de dosage de la DβH utilisant un anticorps lié de façon covalente à des billes de verre. La technique a été appliquée à la mesure de la DβH dans les surrénales et différentes régions du cerveau de bœuf. Les principaux avantages de cette méthode sont sa relative facilité d'emploi, sa précision et sa haute spécificité.

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An Ammoniacal-Silver Stain Technique Specific for Satellite III DNA Regions on Human Chromosomes

Cytogeneticists are currently using in situ hybridization of radioactive complementary RNA fractions to human chromosomes in an attempt to localize the different classes of DNA in specific chromosome regions. Parallel efforts are also being directed toward the development of techniques which differentially stain these chromosome regions. The purpose of this paper is to describe a new procedure, which we call the Ammoniacal-Silver III technique (AS III), that selectively stains those regions of human chromosomes in which satellite III DNA has been localized[1,2]. This method, which is a modification of the Ammoniacal-Silver (A-S) technique[3–7], conspicuously stains the secondary constriction region of human chromosome 9 (Figures 1 and 2). In addition, the centromeric regions of the acrocentric D and G group chromosomes will occasionally differentially stain. Whether the silver binds chemically to the satellite DNA III fraction itself, or to an associated histone or non-histone protein, is not presently known.

When human DNA is fractionated in isopycnic CsCl gradients, it consists of at least 4 satellite fractions[1,2,8,9]. The specific chromosomal locations of satellite I and IV are not known. However, in situ hybridization studies have shown that satellite II DNA is localized in the secondary constriction regions of human chromosome pairs 1 and 16, and possibly 9[10]. More recent radioactive labelling experiments have shown that satellite III DNA is conspicuously concentrated on human chromosome pair 9 and to a lesser extent near the centromeres of all chromosomes of the D and G groups[11,12]. It is these satellite III chromosomal regions that the AS III technique differentially stains.

The AS III stain is prepared by slowly dissolving 8 g of silver nitrate into a solution of 10 cm^3 of distilled water and 10 cm^3 of concentrated ammonium hydroxide. The resulting staining solution is colorless and has a pH of 12.0–12.5. The AS III solution is filtered twice into a vial. 4 drops of the AS III stain are pipetted onto the surface of a microscope slide containing standard air-dried human chromosome preparations from leucocyte culture[13]. 4 drops of 3% formalin (neutralized with sodium acetate) are immediately added to develop the AS III stain. The AS III stain and formalin are quickly

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mixed on the slide surface with a glass rod, and a coverglass is added over the staining mixture which now has a pH of 11.0–11.3. The staining reaction can be followed by examination of the slide with phase contrast microscopy first, followed by bright-field illumination as staining progresses. The chromosomes are usually sufficiently stained within 5 min. If the chromosomes are understained after 5 min, the coverglass may be washed off under running distilled water, the slide blotted dry, and restained. After the proper differential staining is reached, the coverglass is rinsed off and the slide is taken through an alcohol dehydration series, soaked for 5 min in xylene, and mounted in a suitable mounting medium. To make certain that the silver was indeed attaching to chromosome pair 9 and not some other similar chromosome of the C-group, chromosome spreads were first differentially stained with quinacrine, photographed, destained, and restained using the AS III method.

The attachment of the silver stain to the secondary constriction region of chromosome pair 9 is most conspicuous when all other chromosomes of the complement are only lightly stained (Figure 1). Overstaining causes all of the chromosomes to become generally darkened and the differential staining of chromosome 9 is lost.

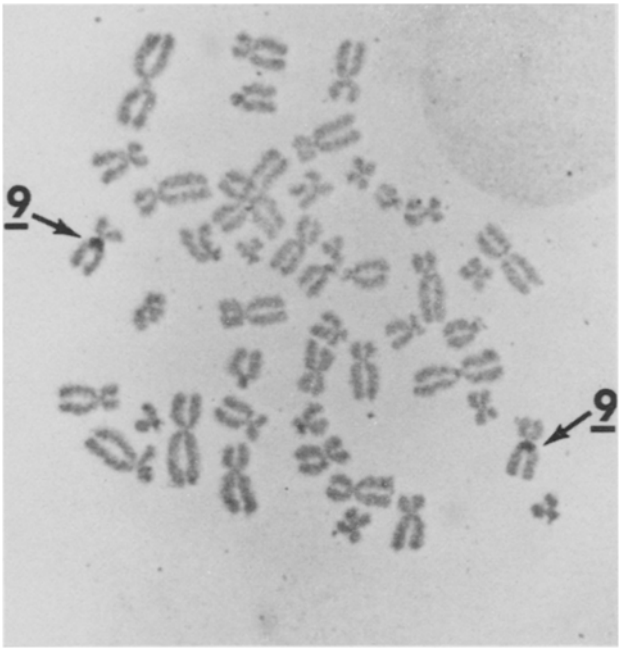

Fig. 1. Human metaphase chromosomes stained by the AS III technique, showing differentially stained secondary constriction region of chromosome pair 9 (arrowed).

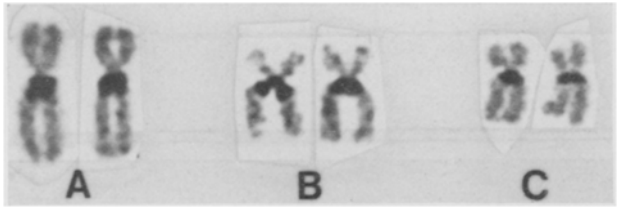

Fig. 2. Three pairs of chromosome 9 showing: A) homologues homomorphic and B, C) homologues heteromorphic for secondary constriction regions.

Black and Speer[3] were the first to use ammoniacal-silver as a nuclear stain. Experiments indicated that their A-S technique specifically stained acid-extractable nuclear protein (AENP) whose physical characteristics corresponded to that of a histone[3–5]. It yet remains to be established whether the AENP was histone in toto or in part. Formalin pretreatment of chromosomes was thought by them to be an obligatory step in the A-S reaction as untreated chromosomes did not stain[3–5]. Our AS III technique differs notably from the A-S reaction in the omission of the formalin pretreatment step and in the proportions of ammonium hydroxide to silver nitrate.

Numerous papers have been published within the last 4 years utilizing various techniques to produce differential banding patterns in human chromosomes. Except for the techniques involving fluorescence banding[14–17], all other procedures[18–24] utilize Giemsa stain. Although we do not yet completely understand the factors involved in the AS III-chromosome interaction, we believe that the cytochemical basis for the differential staining may be similar, if not identical, to that of the Giemsa-chromosome binding. Our basis for this belief comes from 2 lines of evidence: 1. Bobrow et al.[25] recently stained human chromosomes with Giemsa at pH 11 and found that the most conspicuously stained chromosome region was the secondary constriction region of chromosome 9. Thus, it appears that their Giemsa at pH 11 stains satellite III DNA regions. In this respect, the pH 11 Giemsa seems to interact with the satellite III DNA fraction in much the same manner as our AS III reaction, which also stains at pH 11. 2. By varying the pH of the AS III solution, we have occasionally obtained chromosome bands apparently identical to the G-bands achieved with the Acetic Acid-Saline-Giemsa (ASG) technique of Summer et al.[18]. We feel that bandings factors apparently common to both Giemsa and ammoniacal-silver staining are controlled to a large extent by pH. We are presently working to standardize banding patterns equivalent to the G-bands by shifting the pH of the AS III solution.

The cytogenetic significance of a stain specific for chromosome pair 9 is obvious in light of recent findings. Using the secondary constriction of chromosome 9 as a marker, 4 recent papers have shown that pericentric inversion of chromosome 9 is a common heteromorphism of the human karyotype[25–28]. Bobrow et al[25] karyotyped an abnormal child and found a pericentric inversion in one homologue of chromosome pair 9. The child's clinically normal father had the same abnormal chromosome. A similar, if not identical, chromosomal variant was reported by Lubs and Ruddle[29]. It has been suggested

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that chromosome 9 contains a fragile point where the centromeric heterochromatin joins the euchromatin of the long arm[28]. Indeed, cases involving fragility of the heterochromatic segment of chromosome 9 have been reported[30,31]. Obvious length variations are often found in the secondary constriction areas of chromosome 9;[25,27,28,32,33]. Apparently these heteromorphic homologues (Figure 2) segregate normally in pedigrees and should provide valuable data in linkage studies[28].

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We feel that the AS III technique could become a useful procedure for cytogenetic laboratories in the clinical screening of chromosome 9 for possible anomalies. In addition, we hope that the AS III reaction will prove useful to cytochemists in their quest to discover the mechanisms involved in differential staining of human chromosomes.

Résumé. On décrit une nouvelle technique pour les teintures de chromosomes humains. La technique, une modification de l'argent-ammonical, teint sélectivement la région de constriction secondaire du chromosome paire 9, et quelquefois les régions centromériques des chromosomes acrocentriques des groupes D et G. C'est d'un intérêt cytogénétique, puisque les régions chromosomiques sont celles dans lesquelles la fraction satellite DNA III a été découverte par des études d'hybridisation in situ.

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A Pressure Device for Intracellular Injection

An injection device has been developed in which heat is used to elevate the pressure in a micropipette so as to force its content through the tip and into the impaled cell. This technique has the advantage of permitting the injection of both ionic and non-ionic substances and the simultaneous recording of the electrical activity of the cell. Whereas a single pipette suffices for both injecting and recording if the injected fluid is conducting, a double micropipette can be prepared when non-ionic substances are to be injected.

The heating device (Figure 1) consists of a brass tube provided with a rod for fixation to a micromanipulator. The tube accomodates a heating filament (C, Figure 1) supported by a machine screw tightly fitting the upper extremity of the tube. The preparative steps for injection are as follows[1]: a glass micropipette (Corning 7740) partially filled with the solution to be injected (G, Figure 1) is attached to one extremity of a short silver pipe (F, Figure 1) while a piece of the same glass tubing is used to connect the other end of the silver pipe to a drilled machine screw. Solution for injection is then added to the pipette so that it comes into contact with the silver pipe and the connecting glass tube is filled with paraffin oil (E, Figure 1) in order to insulate electrically the pipette from the heating device. The machine screw bearing the mounted pipette is then tightly fitted to the brass tube which was filled before with an excess of alcohol (D, Figure 1). During the assembly of the pipette, care must be taken to avoid formation of air bubbles in any of the liquids used to fill the pipette and the heating device. The entire device is fixed to the micromanipulator and the silver pipe connected to a unity gain electrometer

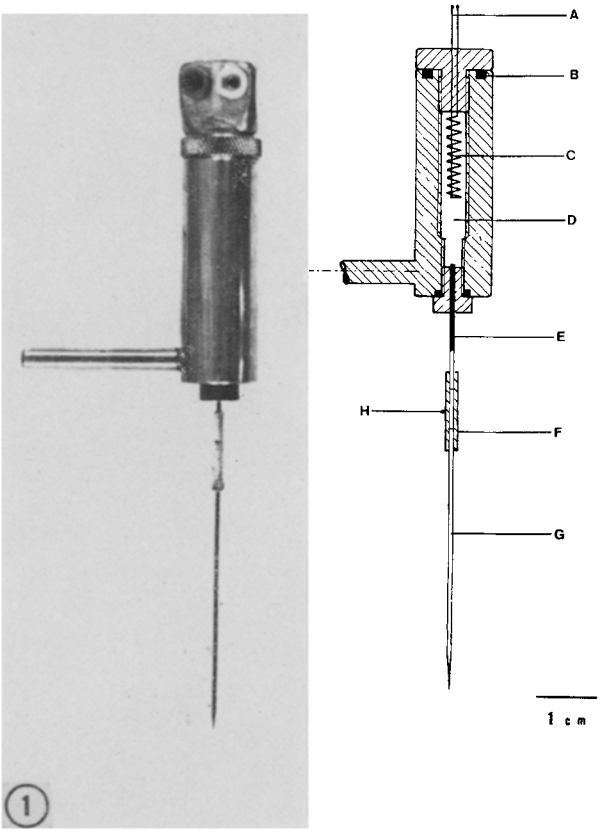

Fig. 1. Pressure device. A) wires connected to the DC current source; B) 0-ring joint; C) heating filament (Ni-Cr-Fe ∅ 0.5 mm 5.7 Ω/m); D) alcohol; E) paraffin oil; F) silver pipe; G) pipette; H) connection for recording.

[1] The mounting of the different elements of the micropipette is carried out with a rapid bounding adhesive (Cyanolit®). This adhesive ensures a leakproof assembly which can be dissolved in acetone and allows the recuperation of the metallic components (silver pipe, machine screw) for making new pipettes.