Field studies of androgen pharmacology are complicated by the necessity to collect, process, and store blood samples in a central facility. We have assessed the feasibility of using capillary blood spots collected by fingerprick and dried on filter paper for pharmacokinetics and pharmacodynamic measurements with nandrolone and testosterone RIAs modified for extracts from capillary blood spots. Assays on punched spots of 7.9-mm diameter (14.9 μL of dried blood) permitted accurate quantification of testosterone down to 0.4 nmol/L from a single spot and nandrolone down to 0.9 nmol/L from two spots. Stability of the steroids in dried blood spots to adverse environmental conditions, notably increased temperatures, was investigated both in the laboratory and in field studies of dried spots sent through the postal system. Storage or postal transport under moderate conditions appeared to have no deleterious effects on apparent androgen concentrations. However, under extreme conditions of storage at 50 °C for a week or more, or transport to a very hot tropical location, a rise in the final concentration of nandrolone, and, to a lesser extent, testosterone when corrected for tracer recovery, was noticed. These effects were largely due to apparent susceptibility of tritiated tracer, but not unlabeled androgens, to thermal degradation. In a pilot pharmacological study involving intramuscular injection of 100 mg of nandrolone decanoate in 1 mL of arachis oil, nandrolone concentrations in concurrently collected plasma as well as venous and capillary blood spots showed good agreement. Testosterone concentrations in contemporaneously collected plasma and venous blood spots also showed very good agreement. We propose that these methods may allow patients and experimental subjects to self-collect samples at remote or field locations for convenient mailing to a central laboratory for androgen assay. Applications of this methodology are now under way.

INDEXING TERMS: fingerprick blood vs plasma sample • androgen • testosterone • nandrolone • radioimmunoassay • sample handling

Field studies in pharmacology and anthropology raise problems of remote sample collection, storage, and transport. Pharmacological studies of long-acting steroids in the field have been limited by the need to standardize blood sampling and sample storage facilities. Adequate sampling to characterize accurately and completely steroid elimination may require prolonged sampling, which necessitates subjects making repeated and sometimes prolonged visits for blood sampling at a centralized clinical research facility.

Rapid, early sequence sampling also requires an indwelling venous cannula and detaining the subject for a period of time. Therefore a trained staff for venipuncture as well as sample handling, storage, and transport facilities is needed. These invasive procedures also artificially confine subjects’ activities, with unpredictable effects on steroid absorption and metabolism, which limits the generalization of such findings to real-world experience. Furthermore, the problems of sample collection, storage, and transport restrict most studies to specialized centers. Studies of prototype androgen-based hormonal male contraceptives have raised questions of ethnopharmacological differences in response of different populations to androgens [1–3].

Recent advances in population pharmacokinetic estimation techniques have the potential to liberalize the stringency of sampling frequency. However, a simpler and less invasive mechanism to obtain blood samples would be highly desirable for such field studies. The major noninvasive alternative to repeated blood sampling has been the use of saliva. Salivary immunoassays have been described for steroids including testosterone [4], 17-hydroxyprogesterone [5], other androgens [6], and
cortisol [7]. However, repeated, frequent collection of saliva may be difficult and suboptimal for remote sampling because salivary samples remain perishable and require handling of bulky tubes. In addition, sonication or centrifugation of samples may increase the apparent concentration of testosterone and other steroids in saliva [8] and, critically, results are invalidated by admixture of even tiny amounts of blood arising from oral abrasions caused by toothbrushing, eating, or dental disease [9, 10].

Schramm et al. [10] and Kathol et al. [11] describe oral diffusion sink (ODS) devices for collection of testosterone and cortisol, respectively. The study by Schramm et al. described oral diffusion sink (ODS) devices for collection of testosterone and cortisol, respectively. The study by Schramm et al. [10] in particular clearly shows that their ODS is designed to circumvent contamination with binding proteins. Dabbs et al. [12] has demonstrated problems of reproducibility between centers in salivary testosterone measurement. Finally, the lack of circulating binding proteins in saliva means that salivary steroid concentrations are very low and reflect only free but not total testosterone concentrations. This makes studies of androgen pharmacology, where suppression of endogenous testosterone may be expected, difficult. Thus, saliva is not ideal for remote sampling studies.

An alternative sample matrix is the filter paper spot. Filter spots have been widely used for large centralized population screening programs for neonatal detection of rare but treatable genetic disorders such as phenylketonuria, hypothyroidism, and congenital adrenal hyperplasia (CAH) [5, 13, 14]. They have not, however, been evaluated for use in steroid pharmacological studies, although a filter spot testosterone assay with limited validation has been reported [15]. We proposed that testosterone and nandrolone concentrations from pharmacological studies could be measured with filter paper blood spots because concentrations would be comparable with those of 17-hydroxyprogesterone concentrations in the screening and therapeutic monitoring studies of CAH [13]. Thus we developed and evaluated a system to collect, store, transport, extract, and measure androgens from 15-μL spots of blood dried onto filter paper. In particular, we evaluated the effects on steroid concentrations under adverse environmental conditions simulated in the laboratory as well as in the field.

**Materials and Methods**

**SELECTION OF COLLECTION PAPER AND SAMPLE COLLECTION**

Two brands of cellulose-based filter papers previously used for blood sampling, paper A (Whatman #3; Selby Scientific and Medical, Lidcombe, NSW, Australia) and paper B (Schleicher & Schuell #2992; Schott-Garsko, Terry Hills, NSW, Australia), were evaluated. Dried blood spots were made up from 50 μL of heparinized, unhemolyzed venous blood either fortified with tritiated nandrolone or separated and then reconstituted after charcoal stripping of endogenous steroids from the plasma. Blood-impregnated and blank spots were extracted in 5 mL of methanol for 2 h, with occasional shaking. The extracts were dried overnight and reconstituted with RIA buffer before assay, either without further processing or after filtration with 0.2-μm cellulose acetate filters (Minisart NML; Sartorius, Göttingen, Germany), or after microfugation for 5 min, with the supernatant then being removed.

Venous blood was collected by venipuncture and plasma stored frozen at −20 °C. In some studies, fresh, unhemolyzed venous blood was spotted directly onto filter paper, allowed to air-dry, and stored with desiccant in sealed containers at −20 °C. Capillary blood spots were collected by fingerprick (Autolet II Lancet; Owen Mumford, Oxon, UK), and the blood spotted directly onto filter paper.

**EXTRACTION OF STEROIDS FROM PLASMA AND BLOOD SPOTS**

Microfuged plasma was extracted by a solid-phase method [16]. If tracer recoveries were required, tritiated steroid in 10 μL of ethanol was added to the plasma and allowed to equilibrate for 1 h at 37 °C before extraction. An aliquot of 50 μL (testosterone) or 250 μL (nandrolone) was applied to a glass Pasteur pipette three-quarter filled with dry kieselguhr (Extrulut®; E. Merck, Kilisly, Victoria, Australia) and the outlet constricted with a 3-mm glass bead. After distribution for 20 min, steroids were eluted with four 750-μL aliquots of hexaneethyl acetate (3:2), applied at 5-min intervals. The pooled eluates were air-dried in borosilicate glass tubes, with gentle warming (~18 h, 30 °C) in a fume hood.

Steroids were extracted from dried blood spots by punching out one or more spots of 7.9 mm diameter and soaking for 4 h in 3 mL of methanol in a 25 × 100 mm round-bottom glass tube at 38 °C with occasional mixing. The spot was then discarded and the extract air-dried overnight at 30 °C in a fume hood. The extract was redissolved in 1 mL of warm assay buffer and then reextracted on a kieselguhr column before air-drying overnight in a fume hood. The extract was again dissolved in 500 μL of assay buffer with duplicate 200-μL aliquots for assay or for liquid scintillation counting for tracer recovery.

**TESTOSTERONE AND NANDROLONE RIAs**

We used an assay buffer of 100 mmol/L PBS, pH 7.2, containing 1.0 g/L swine gelatin (Sigma, St. Louis, MO) and 1.0 g/L sodium azide. Dextran-coated charcoal suspension contained 2.5 g/L activated charcoal (WHO Matched Reagent Program) and 25 mg/L dextran 170 (WHO Matched Reagent Program) in PBS. Steroid calibrators were obtained from Steraloids (Wilton, NH) and made up into stock solutions at 1 g/L in ethanol (Spectrosol grade, Ajax Chemicals, Auburn, NSW, Australia).

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1 Nonstandard abbreviations: ODS, oral diffusion sink; CAH, congenital adrenal hyperplasia; and ED50, 50% effective dose.
stored at 4 °C in a tightly stoppered glass container. [1,2,6,7,16,17-(N)-3H]-Testosterone, 9.25 MBq, specific activity 5.00–6.66 TBq/mmol, was purchased from Dupont Australia, North Ryde, NSW, Australia. [19-3H]Nandrolone, 9.25 MBq, specific activity 1.37 TBq/mmol, was purchased from Amersham Australia, North Ryde, NSW, Australia. Polyclonal rabbit antitestosterone-3-(O-carboxymethyl)oxime-bovine serum albumin (BSA) (SGT-1) from B. Caldwell, Yale University School of Medicine, New Haven, CT [17], used at a final dilution of 1:14 300, had cross-reactivities [defined as relative molar potency at ∼50% effective dose (ED50)] with nandrolone (20.5%), 5α-dihydrotestosterone (26.7%), androstenedione (2.5%), estradiol (0.15%), nandrolone phenylpropionate (0.05%), and nandrolone decanoate (<0.001%). A rabbit polyclonal antibody to 19-nortestosterone 17-hemisuccinate [18–20] used at a final dilution of 1:39 000 had cross-reactivities with testosterone (0.04%), 5α-dihydrotestosterone (0.01%), estradiol (0.01%), androstenedione (0.06%), nandrolone phenylpropionate (11.8%), and nandrolone decanoate (0.3%). The very low cross-reactivity of the nandrolone antibody with testosterone allowed elimination of chromatography before immunoassay and was based on the calculation that the maximum endogenous testosterone concentration likely to be encountered in this study (40 nmol/L) would contribute only a negligible apparent nandrolone concentration (0.016 nmol/L) through cross-reaction compared with the expected nandrolone concentrations of 1–15 nmol/L [21].

Dried extracts were reconstituted in 0.5 mL of assay buffer with intermittent vortex-mixing at 37 °C for 30 min. Duplicate aliquots (0.1 mL) were transferred into 12 × 75 mm glass assay tubes with additional aliquots taken for internal recovery estimation. Steroid stock calibrator (10 μg/L in ethanol, stored at −20 °C) was diluted serially in assay buffer to make a calibration curve (0.5 to 1000 pg of nandrolone/tube). Antibodies diluted in assay buffer and tracer [10 000 dpm/tube; ∼8 pg (30 fmol) of testosterone or ∼30 pg (120 fmol) of nandrolone] were each added in 0.1 mL of buffer. The assay tubes in a total volume of 350 μL were mixed and incubated at 4 °C overnight (16 h) before separation by the addition of 500 μL of ice-cold, constantly stirred dextran-coated charcoal for exactly 15 min at 4 °C. The tubes were then centrifuged (2000g, 30 min, 4 °C) and the supernatant decanted into plastic 7-mL scintillation vials containing 4 mL of scintillation fluid (Ultima Gold XR; Packard Instruments, Meriden, CT). The capped, shaken vials were counted by an automated liquid scintillation counter (RackBeta; LKB, Bromma, Sweden) and RIA data were processed with RIACALC software (Wallac, Turku, Finland).

Assay precision, defined as the within-assay and between-assay CVs, were monitored by the inclusion of control samples containing low, medium, and high concentrations of the analyte. The nandrolone assay had a detection limit of 1.8 pg/tube (equivalent to 0.9 nmol/L) and an ED50 of 64 ± 4 pg/tube (n = 8; equivalent to 28 nmol/L) with tracer recovery of 64.8% ± 8.6% (n = 8). The CVs at low (6.5 pg/tube), mid-range (37 pg/tube), and high (121 pg/tube) concentrations (n = 5 assays) were 66%, 5.2%, and 3.3% (within-assay) and 126%,14.2%, and 3.9% (between-assay). The testosterone assay had a detection limit of 0.9 pg/tube (equivalent to 0.4 nmol/L) and an ED50 of 22 ± 3 pg/tube (n = 5, equivalent to 5.5 nmol/L) with a tracer recovery of 63.0% ± 1.9% (n = 9). The CVs at low (16 pg/tube), mid-range (65 pg/tube), and high (140 pg/tube) concentrations (n = 4 assays) were 86%, 21.3%, and 16.4% (within-assay) and 30.1%,13.1%, and 11.6% (between-assay).

**DATA ANALYSIS**

An unpaired t-test was used to compare the two paper types. Unprocessed, filtered, and centrifuged extracts from each paper type were compared by one-way ANOVA with Dunnett’s multiple comparisons test.

Results of assays in laboratory and field studies were examined with unpaired t-tests or one-way ANOVA with Tukey’s multiple comparisons test.

Data from matched series of plasma and venous and capillary blood spot samples obtained during pharmacokinetic studies were examined by either repeated-measures ANOVA (nandrolone; plasma and venous and capillary blood spots) or a paired t-test (testosterone; plasma and venous blood spots). Additionally, the results obtained for the plasma samples were compared with the corresponding blood spot samples with Bland–Altman plots [22]. Deviation in the Bland–Altman plot was calculated as the difference between the values for the blood spot sample and the simultaneous plasma sample, divided by the mean of the two values. Evidence of bias in the Bland–Altman plots was sought with McNemar’s test [23].

Statistical analyses were conducted with NCSS (Kaysville, UT) statistical software or Instat (GraphPad, San Diego, CA).

**Results**

**SELECTION AND EVALUATION OF FILTER PAPER**

Paper B produced large assay blank interference before blood impregnation that could not be removed by washing with acid, alkaline, or organic solvents (data not shown). The blank interference was partly reduced by filtration but was unaffected by microfiltration (Table 1). Microfugation had no effect on tracer recovery but filtration decreased the recovery of nandrolone tracer from the spots from 84.0% to 57.6% for paper A and from 72.7% to 50.7% for paper B. For all subsequent experiments we used paper A without any additional preparation.

To calibrate the volume of blood impregnated in a filter spot, known volumes of venous blood (30 or 50 μL) were applied to filter paper and air-dried. The area of the blood spot was calculated by cutting out an enlarged copy of the image and weight-comparing with a standard known
Table 1. Selection of filter paper and effects of filtration and microfugation on assay binding and tracer recovery.

<table>
<thead>
<tr>
<th>Filter paper only</th>
<th>Paper A (B/B₀, %)</th>
<th>Paper B (B/B₀, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>99 ± 2</td>
<td>67 ± 3</td>
</tr>
<tr>
<td>Filtration</td>
<td>110 ± 3</td>
<td>87 ± 4</td>
</tr>
<tr>
<td>Microfugation</td>
<td>99 ± 1</td>
<td>71 ± 2</td>
</tr>
</tbody>
</table>

Steroid-free blood spot

<table>
<thead>
<tr>
<th></th>
<th>Paper A (B/B₀, %)</th>
<th>Paper B (B/B₀, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>107 ± 2</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>Filtration</td>
<td>116 ± 3</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>Microfugation</td>
<td>104 ± 4</td>
<td>72 ± 5</td>
</tr>
</tbody>
</table>

Tracer recovery from blood spot

<table>
<thead>
<tr>
<th></th>
<th>Recovery, %</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>84 ± 1</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Filter</td>
<td>58 ± 2</td>
<td>51 ± 1</td>
</tr>
<tr>
<td>Microfugation</td>
<td>85 ± 1</td>
<td>73 ± 1</td>
</tr>
</tbody>
</table>

Effects of paper selection and of filtration or microfugation on tracer binding in the nandrolone RIA and on recovery of tritiated nandrolone tracer. Binding under each condition was compared with binding in the absence of extraction, which was defined as 100%. For details of filtration and microfugation see text. For direct and microfuged samples, n = 6, and for filtered samples, n = 5.

* P < 0.0001, unpaired t-test; ** P < 0.001, ANOVA with Dunnett’s posthoc test.

Stability of androgens in dried blood spots under laboratory conditions

The stability of androgens in dried blood spots during transport was evaluated in the laboratory over 1 or 2 weeks at high temperatures (35 °C and 50 °C) compared with control samples stored at −20 °C over silica gel desiccant. Samples for testing at 35 °C and 50 °C were maintained in closed glass 20-mL vials and stored at the appropriate temperature in drying ovens. The 1- and 2-week experiments were staggered to allow extraction and assay of all the samples together. The uncorrected nandrolone and testosterone values (pg recovered) did not change significantly during exposure to high temperature (Table 2; 35 °C and 50 °C) for up to 2 weeks compared with samples stored at −20 °C over desiccant gel. In contrast, recovery of tritiated steroid tracers from blood spots declined progressively during storage, especially at the higher temperatures, compared with the control spots stored at −20 °C. These effects were more evident for nandrolone than for testosterone. As a result of differential tracer recovery, correcting the androgen concentrations for tracer recovery led to apparent increases in blood spot nandrolone concentration within 1 week of storage at 50 °C but not at 35 °C. The corrected testosterone concentration did not increase at 35 °C and only increased significantly after 2 weeks at 50 °C.

Table 2. Influence of temperature and time on recovery of steroid from filter paper.

<table>
<thead>
<tr>
<th></th>
<th>35 °C</th>
<th></th>
<th>50 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cold, pg</td>
<td>Tracer, %</td>
<td>Conc, nmol/L</td>
</tr>
<tr>
<td>Nandrolone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>69.2 ± 1.1</td>
<td>59 ± 0</td>
<td>34.6 ± 0.6</td>
</tr>
<tr>
<td>Week 1</td>
<td>65.9 ± 2.4</td>
<td>51 ± 1b</td>
<td>38.0 ± 1.4</td>
</tr>
<tr>
<td>Week 2</td>
<td>64.3 ± 2.2</td>
<td>51 ± 1b</td>
<td>37.0 ± 1.3</td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>130 ± 15</td>
<td>64 ± 3</td>
<td>59.8 ± 5.1</td>
</tr>
<tr>
<td>Week 1</td>
<td>118 ± 10</td>
<td>59 ± 0</td>
<td>58.8 ± 3.0</td>
</tr>
<tr>
<td>Week 2</td>
<td>121 ± 6</td>
<td>63 ± 2</td>
<td>56.6 ± 1.7</td>
</tr>
</tbody>
</table>

Response of recovery of cold androgen, tracer, and the calculated concentration of nandrolone and testosterone to storage at two increased temperatures in dry conditions. Results are mean ± SD of duplicates. Changes in recovery or concentration occurred after the first week (Tukey’s multiple comparison test; a P < 0.05, b P < 0.01, c P < 0.001).
assay, together with controls kept at $-20 \, ^\circ C$ in a dry atmosphere in the laboratory.

In the summer experiment, nandrolone content remained stable after return from TV but apparently increased after return from AS. Nandrolone tracer recovery was significantly lower after travel to TV but not AS. Consequently, after correction for blank and external tracer recovery, corrected nandrolone concentrations were significantly higher than controls in spots sent to both destinations. Neither the raw nor the corrected testosterone values obtained after transportation differed from controls, although testosterone tracer recovery appeared to increase after travel to both destinations. The winter experiment, with milder climatic conditions, demonstrated no significant changes in cold, tracer, or corrected values for either androgen (Table 3).

CORRELATION OF BLOOD SPOT WITH PLASMA ANDROGEN CONCENTRATIONS IN A PHARMACOKINETIC STUDY

Venous and capillary blood samples were obtained serially from a single subject participating in a pharmacokinetic study involving the intramuscular injection of 100 mg of nandrolone decanoate in 1 mL of arachis oil (Decadurabolin®; Organon, Sydney, Australia). At each sampling time point, heparinized venous blood was pipetted onto filter paper and capillary blood was collected by fingerprint. Nandrolone (Fig. 1) and testosterone (Fig. 2) results were corrected for blank and external tracer recovery. Blood spot results were corrected for venous hematocrit, to assist in comparing blood spot and plasma results. Testosterone values were corrected for the concurrent nandrolone concentration because of the 20.5% cross-reactivity of nandrolone in the testosterone assay. Both venous and capillary blood spots gave significantly higher nandrolone readings than plasma samples ($P < 0.001$, repeated-measures ANOVA), whereas blood spots from venous or capillary blood gave similar results ($P > 0.05$). Bland–Altman plots showed significant bias only in venous blood spots compared with plasma nandrolone concentrations ($P = 0.0005$, McNemar’s test). Testosterone concentrations obtained from plasma and venous blood spots showed no significant difference ($P = 0.11$, paired $t$-test) nor any systematic bias identified by a Bland–Altman plot.

**Discussion**

This study has established and validated nandrolone and testosterone immunoassays applicable to blood samples collected, stored, and transported on cellulose-based filter papers. These findings suggest that, under moderate environmental conditions, filter spot sampling would be feasible for sample collection and transport of remotely collected serial samples for testosterone and nandrolone.

| Table 3. Influence of travel to and from Townsville (TV) or Alice Springs (AS) on recovery of steroid from filter paper in summer and winter. |
|---------------------|---------------------|---------------------|---------------------|---------------------|
|                    | Nandrolone          | Testosterone        |                    |
|                    | Control  TV  AS     | Control  TV  AS     |                    |
| Summer             |                      |                      |                    |
| Cold, pg           | 25.6 ± 0.3 25.6 ± 1.1 29.5 ± 0.4<sup>a</sup> | 25.8 ± 1.6 30.0 ± 0.8 27.2 ± 0.2 |                    |
| Tracer, %          | 66 ± 0.1 46 ± 3<sup>b</sup> 74 ± 3 | 57 ± 1 72 ± 2<sup>b</sup> 66 ± 0.3<sup>a</sup> |                    |
| Corrected, nmol/L | 20.8 ± 0.3 28.7 ± 1.5<sup>b</sup> 25.8 ± 0.3<sup>a</sup> | 9.0 ± 1.7 10.6 ± 0.7 9.0 ± 0.2 |                    |
| Winter             |                      |                      |                    |
| Cold, pg           | 3.7 ± 0.1 — 3.8 ± 0.0 | 3.2 ± 1.0 — 3.7 ± 0.6 |                    |
| Tracer, %          | 56 ± 8 — 54 ± 1 | 66 ± 7 — 59 ± 0 |                    |
| Corrected, nmol/L | 24.3 ± 0.5 — 25.4 ± 0 | 22.3 ± 0.4 — 19.7 ± 3.0 |                    |

Effect of transporting filter paper blood spots to and from arid (AS) and moist (TV) tropical Australia upon recovery of cold and labeled nandrolone and testosterone, and hence on calculated concentration. Results are mean ± SD of duplicates. Differences of the result for each destination from control were sought with Tukey’s multiple comparison test; <sup>a</sup> $P < 0.05$, <sup>b</sup> $P < 0.01$. Fig. 1. Nandrolone concentrations in plasma, venous blood spots, and capillary blood spots after gluteal intramuscular injection of 100 mg of nandrolone decanoate. Results were corrected for blank and recovery, and the blood spot results were corrected for hematocrit. Both venous and capillary blood spots gave significantly higher nandrolone readings than plasma samples ($P < 0.001$, repeated-measures ANOVA), but the two types of blood spots gave similar results. Inset: Bland–Altman plot of nandrolone concentrations in capillary blood spots compared with simultaneous plasma samples. Distribution about the zero line was not significantly different from even distribution ($P = 0.405$, McNemar’s test).
pharmacological studies were lower than expected. We anticipated that the expected androgen concentrations in dried blood spots for CAH due to 21-hydroxylase deficiency can be expected. These findings are consistent with the observation that androgen concentrations encountered during diagnostic screening or therapeutic monitoring for CAH, both of which have been reportedly achieved with filter spot assays.

Our findings with one commercial filter paper, which demonstrated intractable assay blanking problems, indicate that some filter papers may be unsuitable. Extracts from this paper were refractory to filtration or microfugation. The problem probably resulted from liberation of loose cellulose fibers that nonspecifically adsorbed steroids. With suitable filter paper and an organic solvent extraction, impregnation of blood to cover two 7.9-mm diameter circles containing ~30 µL provided sufficient sample to adequately characterize plasma nandrolone or testosterone concentrations. The nandrolone results were less satisfactory, presumably because of the lower sensitivity of that assay; such limitations might be resolved by more sensitive immunoassay techniques such as nonisotopic time-resolved fluorescence labeling.

Beyond the validity of the filter spot androgen assays, the stability of the samples to storage and transport under adverse environmental conditions is an important issue regarding the feasibility of applying such a technology to field studies. The stability of androgen concentrations recovered from filter spots was analyzed after short-term laboratory storage at high temperatures as well as under actual field conditions of postage to and from destinations in tropical Northern regions of Australia in the summer monsoon season. In the laboratory, under relatively mild, constant conditions of high and dry heat, the nandrolone and testosterone concentrations extracted from blood spots were stable, although tracer nandrolone recovery declined slightly in spots stored at 35 °C and more so at the higher temperature (50 °C). We have been unable to find an explanation for the lower recovery and presumably higher susceptibility of tritiated steroid tracers to deterioration, but it most probably represents a differential thermal stability of tritiated compared with unlabeled steroids. This differential tracer stability indicates that nandrolone or testosterone concentrations should not be corrected for apparent tracer recovery, and for other analogous systems, the tracer stability should be evaluated critically.

The stability of the androgens in blood spots were also studied under actual field transport conditions within the regular postal system. Again we found that unlabeled nandrolone and testosterone were recovered in relatively constant amounts, but recovery of tritiated androgens was reduced, especially at the more extreme adverse environmental conditions. As with the laboratory experiments, correction for apparent tracer recovery led to artifactual inflation of apparent steroid concentrations and should be avoided. In contrast, field transport under milder environmental conditions did not lead to any deterioration in tracer or sample estimates. It is clear that androgen concentrations are stable under normal conditions of storage and transport, but that exposure to extreme conditions over a week or more have detrimental effects on the stability of the steroids, or on the sample matrix, leading to measurement inconsistencies. In general, it appears that if samples can be recovered within a week or maintained at lower temperatures, stability of the androgens can be expected. These findings are consistent with the observation that androgen concentrations in dried blood spots are stable in storage at room temperature for 3 weeks [30], although neither longer duration nor more extreme environmental conditions were studied. The most extensive studies with dried blood spots for steroid estimations [13, 14] involved transport of samples from Alaska to New York, but exposure to high temperature seems unlikely in that setting. Progesterone in dried blood spots was found to be stable for up to 15 weeks under dry conditions at up to 25 °C, and up to 9 weeks at 37 °C, although experimentally produced humidity was very deleterious [31]. Hofman et al. [32] found 17α-hydroxyprogesterone in dried blood spots to...
be stable for up to 9 months at similarly moderate temperatures. In the present study, humidity was excluded as the samples were sealed in air-tight plastic bags, and sealed containers containing desiccant were used for routine long-term storage. However, because in the field humidity can influence dried blood spots in neonatal screening programs for phenylketonuria [33] and hypothyroidism [34], deleterious effects on steroid stability cannot be excluded.

These filter spot techniques were then applied in a pilot study of a pharmacokinetic study of androgens. Concurrent samples of plasma, venous blood spots, and capillary blood spots from a volunteer showed a good correspondence between nandrolone concentrations in the venous and capillary spot blood samples. Capillary blood spot results, however, were not significantly different from the plasma concentrations. The testosterone profile obtained from the plasma and the venous spots showed even stronger agreement. The superiority of the testosterone over the nandrolone assay was presumably a reflection of its greater sensitivity; comparable performance could be expected from the nandrolone assay if its sensitivity could be improved while still retaining its high specificity.

We conclude that, under such field conditions as pharmacological or anthropological studies, impregnation of a cellulose-based filter paper with blood is a potentially useful technique for sample collection, storage, and transport of blood to a centralized laboratory for subsequent androgen immunoassay.

References


